nature

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Going on about race

CONSTANT LAMBERT was an opinionated but highly readable commentator on the musical scene of the 1920s and 30s, of which he himself was a part. In *Music Ho! A Study of Music in Decline*, he wrote thus of Russian symphonic music based on folk origins—'. . . the whole trouble with a folk song is that once you have played it through there is nothing much you can do except play it over again and play it rather louder!' The conflict between those who see a genetic component in white/black IQ differences, and those who do not runs some risk of becoming in science what the folk song became in symphonic music. The latest action replay of the controversy was performed in London last week at the Institute of Biology's seminar on Racial Variation in Man.

The conflict is actually a three-cornered affair between those who say 'nature' dominates, those who say 'nurture' dominates, and those who cry racism. These last who vilify the 'nature' proponents as racists—and they managed a modest demonstration outside the hall last week—should be aware of some dangerous pitfalls.

- It is no good simply decrying the IQ test as such. There is no doubt a substantial degree of disaffection with IQ as a valuable measure of anything useful. Fun can be poked at the skills which it seems to encompass; much can be made of the success of those with modest IQs, and the failures of those with astronomical ones; it can be accused of dividing children at too early an age into sheep and goats, but the fact remains that it is a widely used measuring rod. Further, as Professor Thoday put it in *Nature* last year, 'concerning within-group differences . . . relative performance in IQ tests correlates with relative performance in aspects of education that are highly relevant to success in, and the needs of, modern societies.'

- It is no good denying the existence at present of between-group differences in mean IQ. They are real and it serves no useful purpose to pretend that they are the result of errors of measurement or that they are trivial.

- Lionisation of those who have found a low degree of heritability of IQ is wishful thinking and an utterly unscientific approach. It would do the cause of social responsibility no good to be wedded to an attractive but maybe wrong conclusion.

In short, catch phrases such as 'Smash the racist IQ test' and 'Nurture not nature' are no way to approach the problem. But problem there is. It is difficult to escape the conclusion that those who go on saying that heritability is high are feeding racist tendencies, however inadvertently and seemingly innocently, and are supplying an apparent scientific basis for those who wish to justify racial discrimination. Many scientists would, of course, contend that the evidence on which the present debate is being conducted is not such as could correctly be termed scientific, but the general public, unlikely to appreciate the finer points of the internal squabbles of science, will in the end absorb the message that is repeated most frequently and with the most crescendo.

Educational policy-makers may then be under considerable pressure to introduce some sort of *apartheid* system which tries to partition off by racial group in the interests of uniformity. However neat and justifiable such a system might seem on the face of it, it would be going in the wrong direction in two distinct ways: the educational experience should be more than just intellectual pump-priming, it should now more than ever help to reduce societal divisions; and to educate on the basis of group membership rather than individual capability is to waste the diversity of talent which sometimes seems the main asset of society.

The scientist faced with a discovery which is controversial and maybe unpalatable to some has an awesome responsibility to handle it correctly, and he should be prepared if necessary to devote the rest of his scientific life to ensuring that the proper use is made of the discovery. Would racial segregation in education be a proper use for apparent high heritability of IQ?

It is simply not possible, having unearthed a potentially explosive piece of information to sit on the fence in the name of scientific objectivity. Those who do not take every opportunity to minimise the risk of the subsequent explosion are actually helping it to come about. And those who keep raising the inflammatory issue must be presumed to want to speed the explosion.

A hundred years ago

Mist Bows

ON Sept. 14 I was driving from the Lizard just after sunrise with Mr. Lugg of Manaccan. A thick mist covered the fields and moorland. The tops of the farm buildings and corn stacks and the church towers were visible above the sea of mist which, matted on the ground, gave the entire country the appearance of being covered with snow. About 6.30 A.M. the sun was bright on our right hand, and on the left we saw a halo of prismatic colours forming a distinct circle of rainbow at a little distance from and encircling the shadows of our heads, and only broken where the shadows of our bodies interposed. This appearance lasted for ten minutes, and our shadows with their attendant bow showed brightly against the mist background as we passed hedges and fields, and kept pace with us like "the mist raised from the plashy earth" by the hare in Wordsworth's poem,

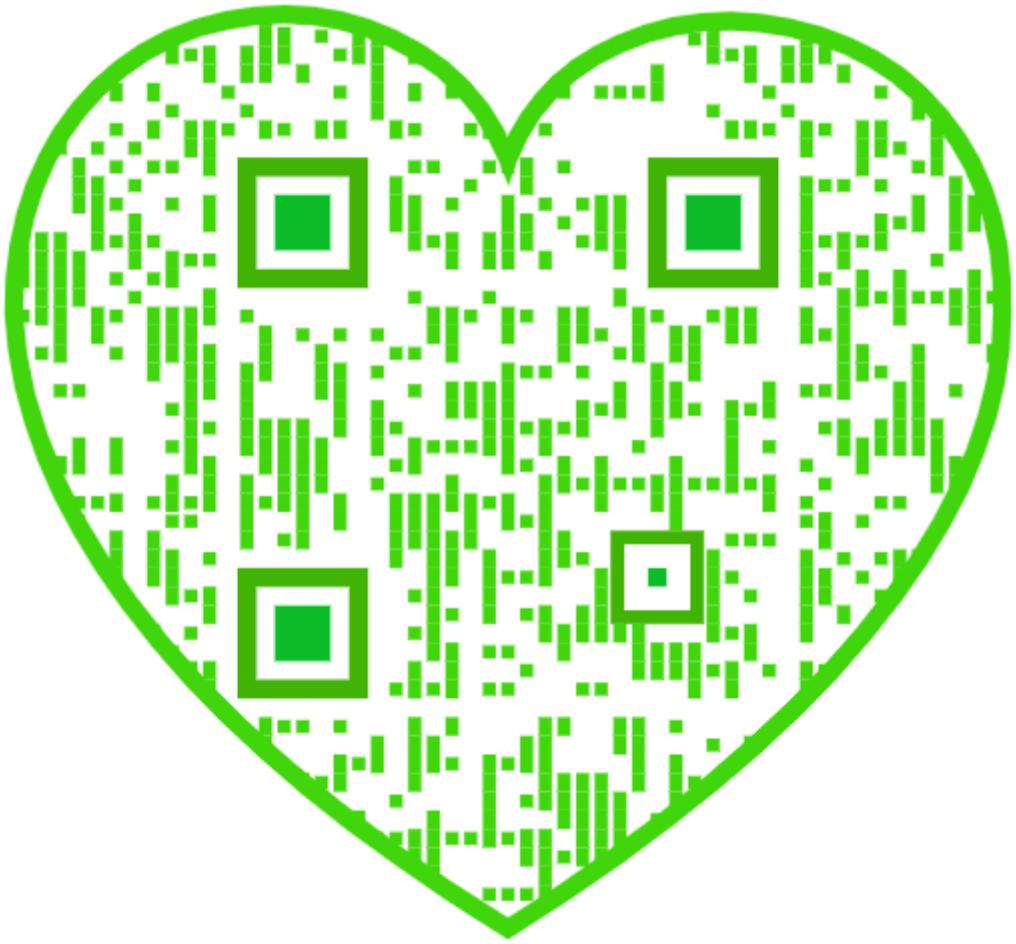
"That, glittering in the sun,
Runs with her all the way wherever she doth run."

We afterwards opened a valley terminating in an extensive moor, when the mist appeared as a sea of prismatic colour extending to the horizon. About 7 A.M. we saw a perfect bow free from any prismatic colour, both ends of which terminated in the field immediately to our left.

My companion, who is constantly driving about this district in early morning, says he never before saw similar phenomena.

Lizard Signal Station, Sept. 16 HOWARD FOX

From *Nature*, **10**, 438, October 1, 1874.



Towards a European Space Agency

SINCE April 1 this year we have awaited the establishment of the European Space Agency (ESA). The new single organisation will take over the functions of the three existing bodies—ESRO, ELDO and the ESC*—to become the NASA of Western Europe. ESA will be responsible for running not only the scientific and application satellite programmes of ESRO but also the three new ventures decided a year ago by ten European space nations—the Spacelab orbital laboratory, the Ariane launcher and the Marots maritime satellite. Each of these three new programmes will be developed under the respective sponsorship of Germany, France and the UK. The rules of participation in the programmes will also be changed by the new convention of ESA. As before, a participating country will have work allocated to it by the Agency in proportion to the amount of cash it subscribes. But now member-states will also have the right to contribute such sums as they feel necessary, and to opt out of specific Agency programmes which they don't find attractive.

The creation of ESA has been progressively delayed throughout this year. The ten European ministers will meet again this week in Paris, but a French government source said last week that there would be no decision until mid-October. Indeed, it is absolutely impossible to foresee what could happen next month. There may be another postponement, but it is hoped that ESA should be settled before the end of the year.

The delay is caused by a fight for the leadership of ESA between the two most important contributing countries. France and Germany have both proposed a candidate as first general manager of the organisation. France is also trying to obtain the participation of the other member states to the cost of the French Guiana Space Centre, which became the launching range for the Ariane rockets. Unexpected events like the sudden death of President Georges Pompidou and the following election campaign have also contributed to the delay.

The new French government hasn't decided much about space affairs—apart from signing the Memorandum of Understanding for the joint development of the Aerosat satellites by Europe and the United States.

On current thinking, the French government should define its space policy in early October at a special council of the ministers concerned. Some official sources say it will closely analyse the space report, and the Ariane project will certainly be questioned. The former government always thought that the heavy launcher was to be "the keystone of the European space policy", considering the political and economical value of the application satellites. More than 3,500 million francs have been spent to duplicate the US heavy rocket that failed when the Europa 2 rocket blew up in the Atlantic. Now France and Germany require an American launcher to put the Symphonie communications satellite into orbit in December of this year. But the US State Department imposed such limitations to the use of this experimental satellite that its interest will be significantly reduced.

Now comes the second and decisive stage for the European rocket. In May, 1973, France proposed to jump a step with Ariane, a more powerful launcher able to place a 750 kg payload into geostationary orbit, which could be developed before the end of the decade. France picked up at least 65% of Ariane costs, which are currently budgeted at about 2,500 million francs for the development only. Ten European countries, including the U.K., are participating in this programme. Tooling up, long-term supplies and engine ground testing are now under way, and the industrial team has been selected recently.

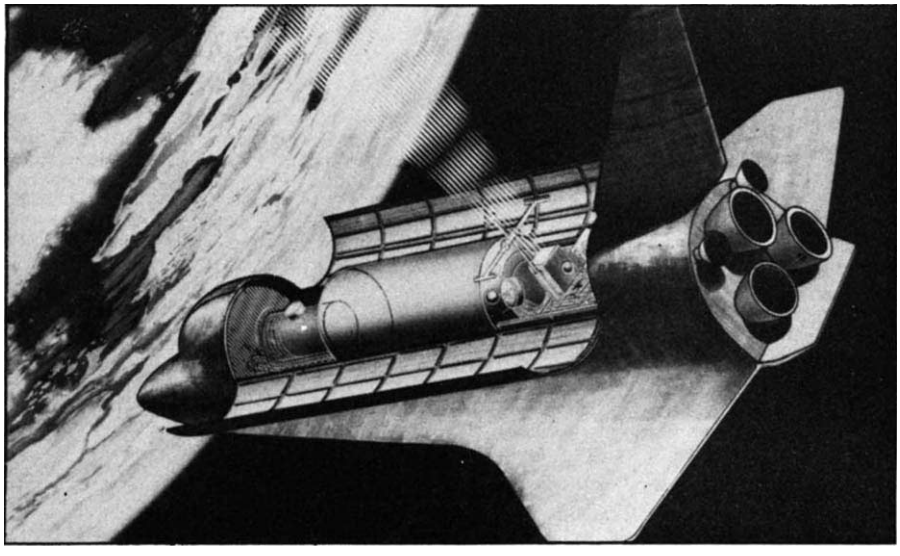
Meanwhile, last June, the French government withheld authorisation for the Centre National d'Etudes Spatiales (the technical and financial manager of Ariane) to conclude the long-term contracts of development with the main contractors. The government needs to think carefully before acting because the programme necessitates more and more spending during the next three years at the same time as the other European space activities also begin to be more costly. In spite of all these new expenses the government don't want to increase the space budget; recent reports from the French Ministry of Industry are very clear about this. In such circumstances the CNES may be obliged to reduce, postpone or even to cancel some of its activities although usually France considers that European expenses take priority.

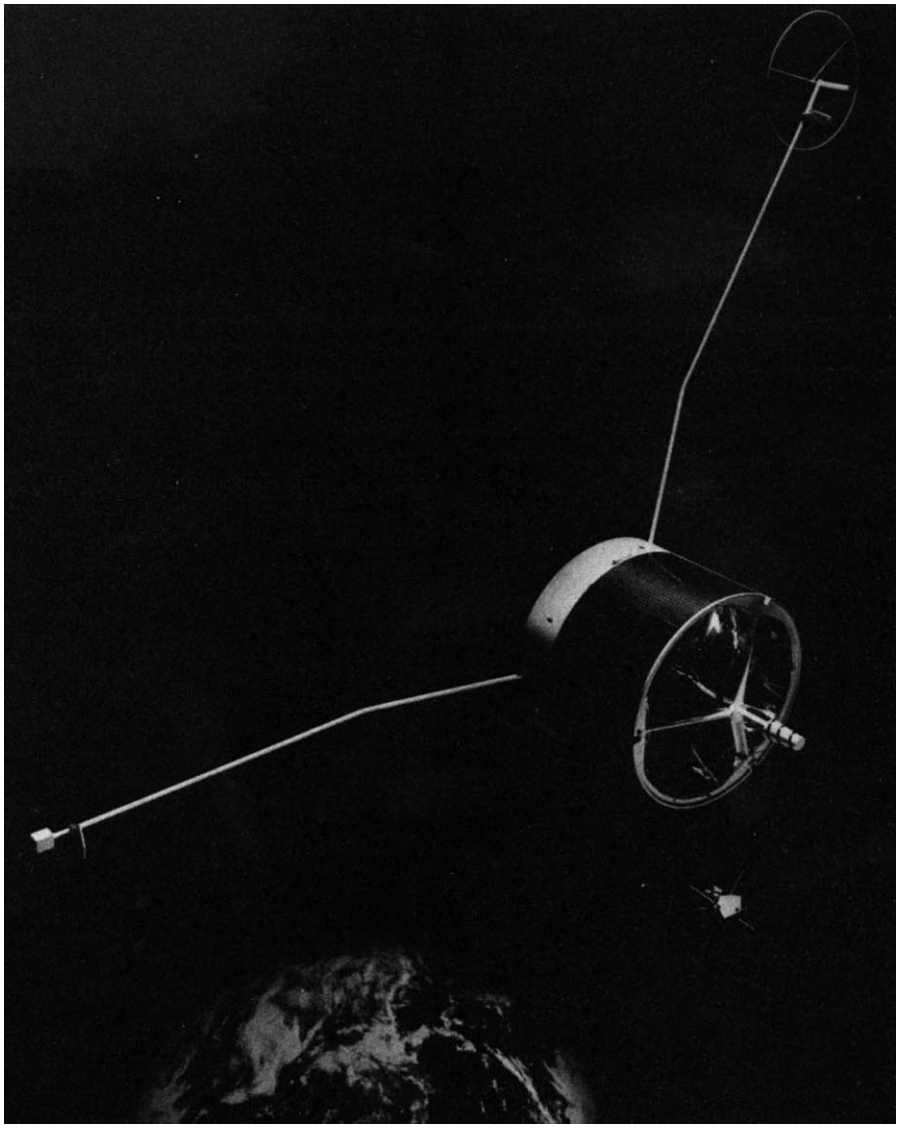
Nevertheless, the Ariane programme could be in danger. The 200-ton three-stage launcher will be the largest rocket built in Europe. But it will be relatively expensive and its market seems less important than expected. A joint study by French and Germans anticipated 35 to 50 launches in ten years (1980–90). But it was never published, and the specifications for the launch range consider only two firings per year during the same period, i.e. less than half the forecast.

The real reason for the difficulties is a political, economical and cultural one: the French desire to build "the launcher of European independence". But it is not certain that the new government will follow its predecessor on this policy. President Valery Giscard d'Estaing has based his policy on change, and on the evidence of the past three months he has a habit of making surprising decisions. For the present government to drop the idea of backing the launcher would be the worst thing possible for the French aerospace industry.

Despite these troubles, the other programmes are running well. Spacelab, which will also be a long-term develop-

Spacelab: first flight planned for 1980.





International Magnetospheric Explorer (IME): to be placed in orbit in 1977.

ment with significant spendings, is going on. This 400 million dollar programme will be a truly international space venture under the leadership of Europe. Developed by 10 countries—all 10 members of ESRO except Sweden but with Austria—the Spacelab will be a part of NASA Space Shuttle Transportation System. European industrial and technical teams are in permanent contact with NASA officials over the development of this spacecraft, which will be completely dependent on the Space Shuttle Orbiter.

It is a versatile manned space laboratory in which scientists and engineers with only a short training as astronauts will be able to live and work for periods of one week to one month in weightlessness. The crew will perform different tasks: scientific missions, Earth resources and weather surveys, communications and other technology experiments. The orbital laboratory has two main parts: a pressurised module connected by an airlock to the Orbiter crew cabin and a pallet which supports instruments exposed to the vacuum of space.

NASA has ordered two Spacelabs from ESRO. The first flight of Spacelab is planned for April 1980, with the seventh flight of the Shuttle, on a mission not yet defined. More than 200 experiment proposals have already been received by ESRO from the European scientific community for this flight.

The next two European scientific satellites will be in space within a year. The COS-B satellite will be launched in April or May 1975 to study gamma rays, and the GEOS satellite will be put into the geostationary orbit in September, 1976, to investigate plasma distribution, energetic particles and electromagnetic fields.

In December, 1976, NASA will launch the International Ultraviolet Explorer satellite (IUE), a joint US-UK project in which ESRO participates. The next project will also be a cooperative one between NASA and ESRO. The ISEE satellite (formerly IME-D) is due for launch in September, 1977, with a brother American satellite from the same launcher to study the magnetosphere. For mid-1979, ESRO is preparaing the highly eccentric lunar occultation satellite (EXOSAT) that will map the X-ray sources.

Since 1970 13 scientific mission proposals have been submitted to ESRO, but only one or two will be retained in early 1976 for development. All these missions could be realised with the Spacelab or free flyer satellites. Two-thirds of them are cooperative projects with NASA. Nine are devoted to infrared, ultraviolet and X radiation studies of the Sun and the sky. Three others are more impressive projects including the first probe to make observations from out of the plane of the ecliptic.

There is also a Pioneer–Jupiter orbiter probe and the interception of Comet Encke by the end of 1980 to proceed with the first close study of thesem ysterious space objects. Another proposal is an astrometry mission to chart more than 30,000 stars down to magnitude 17 with a precision 10 times better than the existing references. The feasibility of this project will be discussed at a special colloquium to be held next month at Frascati (Italy).

Among the application programmes, the meteorological satellite (Meteosat) will be put into geosynchronous orbit in December, 1976, to participate in the Global Atmospheric Research Program of the World Weather Watch. Three other experimental satellites are due for launch close to the end of 1977 to prepare the operational systems of the 1980s. The OTS satellite will pave the way for the European Communications Satellite (ECS) designed to provide space links for intra-European telephony, telegraphy, telex and TV relay for the European Broadcasting Union. The aeronautical satellite (Aerosat) will be the first step towards a world-wide air traffic control and communications satellite system. And the maritime satellite (Marots) will carry out experiments involving communications between ground and merchant ships to prepare the future IMCO operational system to cover the seas and the oceans of Earth. All these new European satellites—like the seven built over 10 years by ESRO—will be launched by American rockets from the United States.

*ESRO is the European Space Research Organisation; ELDO is the European Launcher Development Organisation formerly responsible for the launcher Europa 2, and ESC is the European Space Conference, i.e. the forum for decisions by the European science ministers.

—From the staff of La Recherche

international news

Fruits of a Faustian bargain

by Colin Norman, Washington

CHARLES COLSON, former President Nixon's one-time hatchet man, announced soon after the 1972 election that the Administration would take no vindictive action against those who had the temerity to oppose it. In fact, said Colson, a large federal programme was already in the works for the only state which had cast its votes for McGovern —a nuclear waste repository would be built in Harvard Square.

Fortunately, even though members of the infamous White House enemies list were heavily concentrated in the Boston area, Massachusetts won't be so lucky. That particular federal programme will go to Nevada, Idaho or Washington state.

Last week, the Atomic Energy Commission announced that sites in those three states have been shortlisted as potential locations for a temporary storage facility where highly radioactive waste material from nuclear power stations will be kept under surveillance until a safe, proven method is found to get rid of the stuff. The agency also acknowledged that it has yet to find such a method.

The announcements and acknowledgements were contained in an unusual document—an environmental impact statement in which the AEC, instead of analysing the consequences of a single, chosen programme, has floated some ideas, asked for comments on them, and said that it will take the comments into account when it makes its final choice.

Although that piece of before-the-act public consultation has been welcomed by the AEC's friends and foes alike, it will, nevertheless, do little to dampen the fierce debate over nuclear waste disposal which has been going on in the United States for several years. But it should at least help to sharpen the issues. For those who have tuned in late, a little background is in order.

The waste material in questions consists of radioactive isotopes formed in a reactor core from uranium fission. When irradiated fuel rods are removed from the reactor they contain not only unwanted isotopes such as strontium-90 and cesium-137, but also large quantities of plutonium and unused uranium which are so valuable that they are removed in a reprocessing plant and later reused as reactor fuel.

What is left is an acidic solution of waste material which contains virtually all the radioactivity from the spent reactor fuel and which must, somehow, be disposed of. Since such solutions are not the easiest things to store—in fact, there have been some serious leakages from storage tanks at AEC facilities in Washington and Georgia—the AEC began a programme in the 1950s aimed at finding a method of reducing the waste solutions to stable solids.

A method has been developed which essentially consists of depositing oxides of the unwanted isotopes on granules of aluminium oxide, and in 1970 the AEC issued a regulation which requires that all highly radioactive wastes from commercial power stations be solidified and sealed into steel canisters before being shipped to a federal nuclear waste repository.

The problem, though, is that no such repository yet exists because the AEC has had considerable trouble finding a suitable dumping ground. The trouble stems from the fact that because the reactor wastes contain extremely long-lived radioactive isotopes which will remain highly dangerous for several centuries, they must be kept isolated from the environment for thousands of years.

The most promising disposal method, according to virtually every committee and commission which has studied the matter—including the National Academy of Sciences—is to bury the waste canisters in bedded salt, which is geologically stable, provides an effective radiation shield, and is unlikely to be in contact with subsurface water.

During the 1960s, the AEC found what looked like an ideal site—a disused salt mine near Lyons, Kansas—and in 1971 the agency asked Congress for funds to conduct pilot-scale operations there with a view to turning the mine into a federal repository if all went well. Fortunately, though, the project met with political opposition, Congress denied the funds and it later turned out that the area was, as one Kansas state engineer put it, "like a piece of Swiss cheese". Back to square one. Then, in 1972, the AEC announced that it was going to buy some time by constructing a storage facility above the ground in which wastes could be kept under constant surveillance while a search is made for a permanent disposal site. And that, essentially, is where last week's environmental impact statement comes in, for the AEC has now not only shortlisted potential sites, but it is also close to choosing a design for the storage facility.

In the meantime, however, critics of nuclear power have seized upon the AEC's difficulties and turned them into a potent argument against the burgeoning growth of nuclear generating capacity. They have argued that it is immoral and unwise to push ahead with a technology which will leave a dangerous legacy for many generations. The nuclear power programme should therefore be halted at least until a safe waste disposal method is found and tested. Needless to say, the AEC does not agree.

According to the environmental impact statement, the above ground storage facility will be capable, if necessary, of maintaining waste canisters safely for "at least 100 years", although, with luck, that will not be necessary for the AEC hopes to have found, tested and prepared a permanent disposal site within two or three decades.

The AEC's strategy, as outlined in the environmental impact statement, is this: It will probably choose a design and a location for the storage facility in the next few months, perhaps even in time to ask Congress for construction funds in the 1976 fiscal year budget. The facility should be ready to receive its first shipments of waste canisters by 1981, and it will gradually be extended to cope with increasing volumes of wastes towards the end of the century. By the year 2000, in fact, nuclear power stations in the United States will have produced almost a quarter of a million tons of solidified wastes.

In the meantime, the agency will continue searching for a geologically stable burial site where the wastes can be disposed of permanently. Other salt mines in Kansas, and salt and potash deposits in New Mexico are under consideration, and according to Dr Frank K. Pittman, Director of the AEC's Division of Waste Management and Transportation, some pilot-scale tests could begin at one or more potential

dumping sites in the 1980s. Pittman added, however, that although he is very hopeful that a suitable site will be found, the AEC has no "implicit faith" that such a site exists.

As for the temporary storage facilities, the environmental impact statement describes three potential designs, each of which has its own advantages and disadvantages. In terms of cost, however, they would work out at about the same—$100 million.

The essential feature of any such repository is that the waste canisters must not only be shielded for radioactivity, but they must also be kept cool because the radioactive decay gives off vast amounts of heat—the temperature at the centre of the waste canister could reach almost 1,000° C. One of the designs would use water as the coolant, while the other two would rely on air cooling.

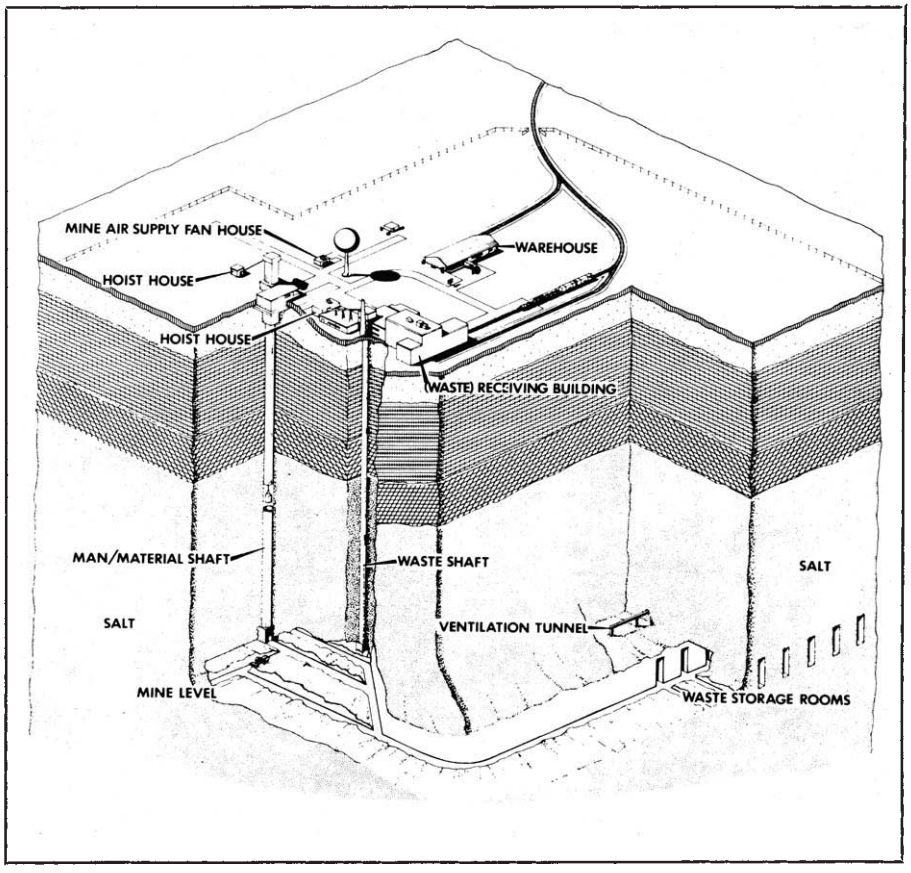
- The water-cooled design would consist of a series of stainless steel-lined concrete vaults, each capable of holding about 500 canisters and each filled with demineralised water. The water would be kept cool by continuous circulation through a closed loop in which the heat is given up to a secondary cooling loop and dissipated to the atmosphere through a cooling tower. As well as providing cooling, the water also acts as an effective radiation shield, and since it will be constantly monitored for radioactivity, canister leakage can quickly be spotted.

The disadvantage with this design, however, is that it would require large quantities of cooling water, and corrosion may also be a problem over long periods of time.

- One of the two air-cooled storage designs under consideration—the so-called air-cooled vault concept—would consist of a series of large vaults similar to those in the water basin design, each of which would be capable of storing 500 canisters. Each canister would, however, first be sealed in a steel overpack to provide extra protection against the canister rupturing and spewing out its radioactive contents. Cooling would be provided by passive circulation of air through the vault, which would maintain the temperature at the surface of each canister overpack to about 200° C, and the thick vault walls would provide radiation shielding.

The advantage of this design is that there is virtually no possibility of breakdown in the cooling system, but it is the most expensive design and it would disturb large amounts of land.

- The third choice, the sealed cask storage concept, differs from the other two in that no storage vault is required. Each canister would be sealed in a two-inch thick steel cask which would be surrounded by a 38-inch thick concrete shield to provide radiation protection. A six-inch air space between the concrete and the steel cask would allow cooling air to circulate, which would keep the temperature of the cask to about 120° C. The massive concrete storage units would be kept outside, and by the year 2010 the entire storage area could occupy about 1,100 acres of land.



Bedded salt pilot plant: most promising disposal method

The advantages of this design are, again, that no mechanical cooling system is required, but the disadvantages are the large amounts of land needed and the fact that in the unlikely event of a container rupture some radioactive wastes would be lost to the atmosphere.

The three potential designs are now being looked at by various groups, including a committee of the National Academy of Sciences, and when a final choice is made, the AEC will publish another, more comprehensive environmental impact statement.

The choice of design will, to some extent, also affect the choice of possible site. The AEC has indicated that it is now considering potential sites at the Hanford Reservation in Washington State, the National Reactor Testing Station in Idaho, and the nuclear test site in Nevada. Each of those would be suitable for the two air-cooled designs, but because of the shortage of water the Nevada site would be unsuitable for the water basin design.

In the meantime, the AEC has also been examining a few so-called unconventional waste disposal methods, such as burial in polar ice sheets, placement on the seabed, discharge into volcanoes, or loading the wastes on a rocket and firing them into the Sun. A study conducted by Battelle laboratories has concluded that none of those concepts presents a viable alternative to burial in a stable geological formation such as bedded salt.

Even if the storage facilities will, as the AEC claims, pose little radiation risk to people living close to them—even in the event of the "maximum credible accident" of a canister rupture during storage or handling—the plans outlined in the environmental impact statement are sure to meet with considerable opposition.

For one thing, large numbers of shipments of waste canisters will be required—about 600 a year by 2010—to transfer wastes from reprocessing plants to the storage site. It was, in fact, this aspect which raised most of the opposition in Kansas to the proposed repository at Lyons, and there is no reason to expect that attitudes will change.

But the most powerful argument, which is already being resurrected, is that since there is absolutely no guarantee that a permanent disposal site will be found, the entire nuclear power programme represents a Faustian bargain in which relatively short-term energy benefits have been traded for a very long-term pollution problem. □

Court ruling has its dangers

by Colin Norman, Washington

THANKS to a little-noticed ruling of the Federal Appeals Court in Washington DC, research proposals contained in any grant application submitted to the federal government must be disclosed to anybody who asks to see them—including another scientist working in the same field.

Since grant applications, particularly those submitted to the National Institutes of Health, usually contain a fairly detailed description of the proposed experiments and how they will be carried out, several scientists reacted with some alarm when informed of the court decision last week. And, within NIH itself, there is a general feeling that the ruling will affect the way in which grant applications are written and reviewed.

Brought by a public interest law group called the Washington Research Project Inc., the court case itself involved eleven specific grant applications for research into the effects of psychotropic drugs on children with learning disabilities.

A lower court judge ruled in November last year that the Freedom of Information Act (FIA) requires that not only the grant applications but also the reports of peer-review groups which rate them according to their scientific merit, should be made public. The Appeals Court decided last week, however, that while the grant applications should, indeed, be publicly available, the confidentiality of peer-review reports should be preserved.

A fight is now likely to ensue over exactly how the ruling should be interpreted. Although a final determination had not then been reached, government lawyers suggested last week that since the eleven grant applications in question had all been approved and funded by the National Institute of Mental Health, the decision probably does not apply to applications which are still pending. But a lawyer for the Washington Research Project, citing language in the decision which refers to "approved or pending" applications, argues that it does.

In any case, even if the ruling does apply only to approved grants, it would still cause a radical break from established NIH practice and hold some important implications.

First, there is the possibility of plagiarism of research ideas. According to the Appeal Court judge, the government lawyers took "pains to argue that biomedical researchers are really a mean-spirited lot who pursue self-interest as ruthlessly as the Barbary pirates did in their own chosen field". They argued, in fact, that a scientist would be able to get complete details of a competitor's experimental designs simply by asking NIH for them, conducting the experiments himself, and getting into print with the results before the hapless originator of the idea had been able to do so.

There are, however, rather more subtle ways in which the decision could influence the course of scientific research. It has been argued that one result will be that grant applicants will be less than explicit in giving details of their proposed experiments. Not only would that make the job of scientific review committees more difficult, but it could also favour those scientists who have an established track record.

Another possible consequence, cited by the Association of American Medical Colleges in a court brief, is that "pharmaceutical companies could periodically review NIH and NIMH files for the purpose of converting research findings to commercial use, thereby converting the government into a conduit of free research and methodologies for private gain". Furthermore, sinc the drug companies would also be made aware of what research the government is funding, they would be less inclined to invest in those areas, "thus discouraging independent biomedical research", the AAMC argues.

On the other side of the coin, however, it can be argued that taxpayers have a right to know exactly how the federal government is spending their money, and it would be contrary to the spirit of open government embodied in the Freedom of Information Act to keep research applications confidential.

Be that as it may, the government will probably appeal the case to the Supreme Court, although no decision has yet been made on that score. Alternatively, Congress could attach an amendment either to the Freedom of Information Act, or to other legislation such as appropriations bills, to preserve the confidentiality of grant applications. A move by Senator Robert Dole earlier this year to attach just such an amendment to the Freedom of Information Act was, however, turned back pending the court decision, and Congress is, in any case, unlikely to pass such a measure without first giving it some study at the committee level. □

Israeli jobs for Soviet scientists

from Nechemia Meyers, Rehovot

WILL Israel be able to absorb the 60,000 Jews a year, many of them engineers and scientists, who will reportedly be permitted to leave the U.S.S.R. under the terms of an unpublished agreement between the U.S. government and the Soviet government? To be sure, there is no shortage of jobs in Israel. The building trades, agriculture, industry and various services could absorb thousands of newcomers each year. However, positions are not so readily available for university graduates. Even now, according to Absorption Ministry Director General Menahem Sherman, 1,400 are without immediate prospects of a job.

No one knows how many scientists and engineers will be allowed out, but a large number could appear on the Israeli labour market in a relatively short period of time. Indeed, there are more Jewish scientists and engineers in the Soviet Union—an estimated 100,000—than in Israel, and Israel, of course, has an infinitely smaller economy in which to absorb them.

Israel's National Council for Research and Development has already established a special committee to approve Government allocations to new research units based primarily on immigrant scientists. Groups recently established with NCRD aid include one designed to foster the industrial utilisation of Negev Desert chemicals, another working on tools using radiation for inspection purposes and a third in the field of biomedical engineering.

Dr Vladimir (Ze'ev) Zaretskii (until 1971 a member of the Institute for the Chemistry of Natural Products of the U.S.S.R. Academy of Sciences in Moscow, and now in charge of the Weizmann Institute's Organic Mass Spectrometry Centre) believes that a large number of his former colleagues can be absorbed and help Israel's development at the same time.

"Their place," he says, "is in industrial research. Thousands upon thousands of talented Jewish scientists and engineers work at centres of applied science. Their talents could be utilised by existing Israeli companies or by specially created new ones."

While welcoming the NCRD bodies, Dr Zaretskii believes an even closer link should be forged between industry on the one hand, and immigrant scientists and engineers on the other. He suggests partnerships that would bring together the research knowhow of the Russians with the business knowhow of Israelis and Americans. "People trained in the U.S.S.R. don't understand Western concepts of profit margins and marketing. But they do know how to work," Zaretskii says.

Certainly this isn't the best time for industrial expansion. Israel's resources are strained to the limit and beyond by defence needs. Moreover, it is becoming increasingly difficult to export goods at a time when world markets are shrinking and country after country is sliding into a recession. □

Soyuz-15: inside information

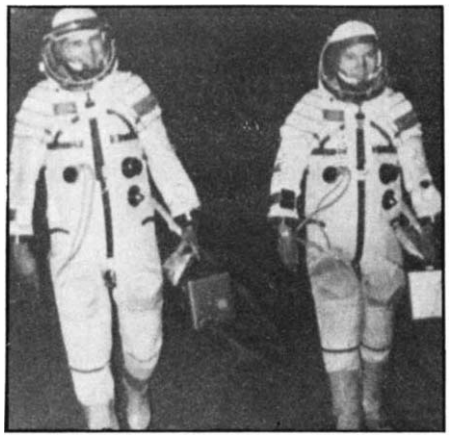
by John Gribbin

THE abortive flight of Soyuz-15 provided valuable tests of an automatic docking system intended for use by future "tanker" spacecraft. That at least is the inside story being put about at the Johnson Space Center by Major General Vladimir Shatalov, the Soviet cosmonaut chief (*Aviation Week & Space Technology*, September 16, 22; 1974).

Shatalov has been visiting the Apollo-Soyuz Test Project crew training sessions, and took advantage of the opportunity to give more details of the official Soviet view of Soyuz-15. Confirming that the spacecraft never made physical contact with the space station Salyut 3, he said that the automatic rendezvous system repeatedly took the spacecraft to within 30 to 50 m of the space station, but that the final rendezvous sequence failed each time.

According to Shatalov, docking using manual control would have been possible on any of these occasions. But that was not the purpose of the mission, and the Soviets preferred to spend the available time in repeated tests of the automatic docking system. Time was limited, apparently, because the Soyuz-15 mission was from the outset planned to last only two days, and was intended to land at night to test techniques. Even if docking had been achieved, it seems that the cosmonauts would have undocked and returned—after further docking tests—without entering the Salyut.

The official view is that the flight was a success because it tested the automatic docking system. This system will be needed in future developments of the manned space programme, when "tanker" derivatives of Soyuz will be used in an unmanned configuration to ferry fuel and other supplies up to space stations.



Soyuz-15 crew: could have docked

In the USA, there is some scepticism about Shatalov's outline of the Soyuz-15 mission. It is felt very unlikely that two men would have been sent to dock with Salyut-3 without any intention of their entering the space station, and it is also pointed out that the very first docking of two Soviet spacecraft involved unmanned vehicles, and was accomplished seven years ago. Although it seems unlikely that Soyuz difficulties of the kind experienced by Soyuz-15 are related to the joint programme, Senator William Proxmire has expressed concern and says that "present plans for a joint space mission in 1975 should be seriously re-examined in the light of the continuing difficulty in the Soviet program".

But perhaps his fears—and those of others—might be at least partly allayed by two other items of news made public last week. General Shatalov has publicly thanked his US counterpart, Brigadier General Thomas P. Stafford, and US astronauts for not revealing details of Soyuz-14 prior to the launch. It seems that the names of the cosmonauts and the date of launch were known to US astronauts training in the USSR last July, but that at the request of Soviet officials they said nothing to the press "in consideration of Soviet traditions and customs".

That, according to Shatalov, demonstrates the mutual trust needed to make the joint mission work. The other news, however, concerns more tangible preparations. Shatalov has now confirmed that at least one manned mission will be carried out to test Apollo-Soyuz hardware, about two months before the planned date of the joint mission, which is next July. This proving flight can only be carried out by the Soviet team, since NASA only has one Apollo/Saturn vehicle left, to use on the joint mission itself.

300 universities look to the future

from a Correspondent

THE General Assembly of CRE meets once every five years; often enough, it might be thought by those to whom the sight of one Vice-Chancellor is daunting enough, let alone 300. The theme of the Assembly at its meeting recently in Bologna was "The European Universities 1975–1985", and the Assembly divided itself into five groups to discuss particularly the University and the Changing Needs of Society, University Training, University Research, University Government, and the Financing of Universities.

After four half-day sessions the whole Assembly met to hear reports from the five discussion groups. With one or two honourable exceptions, the working papers which had been prepared before the Assembly could only be described as lead balloons, but a combination of good discussion in the groups and excellent work by the chairmen and rapporteurs led to useful and sensible final reports.

The report of the first study group (the University and the Changing Needs of Society) stressed particularly the difficulties that the universities have in responding to the needs of society when those needs are only imperfectly articulated in the present, to say nothing of needs in the future. This made it all the more important that universities, so far as they can, should give not only academic advice to students but should help them with the role they were later to play in society. Since migration in the field of academic professions is growing, an international understanding of the equivalence of diplomas and degrees needed to be established, although the Assembly was very much aware of the danger for the "Third World" of losing some of their best people to the developed countries. While the university should be responsive to society's demands, it should keep constantly before itself its special role as "the place where the most complex and profound work can be done". Perhaps the surprise of this session (at least to Western Europeans) was the "points" system of university entrance practised in Eastern European countries and described by the Rector of Warsaw University, where points could be granted not only to first-generation children of peasant origin but also to winners of sporting events. In Poland and Czechoslovakia, for example, winners of events in national Olympic games are exempted from university entrance examinations.

There was considerable disquiet expressed in the discussion group on University Research about the squeeze on funds for research as financial pressure on universities increased, and how important it was to explain to the public and politicians the essential role of research in universities.

So far as international research projects were concerned, although these were generally to be welcomed, they could be successful only if there was a genuine scholarly need for them. In some cases (and CERN was cited as an example) these projects had been outstandingly successful, but this had not been uniformly the case. Sponsored

research was discussed at some length and with enthusiasm. The group felt, however, that there were two basic conditions which must be met by such research: the first was that it must be stipulated that the scientific content of such projects should always be published; the second was that sponsored research should not interfere with the general research policy and the teaching duties of the university.

In the discussions of the financing of universities it was emphasised that, although universities needed to know basic data—such as staff/student ratios, number of square metres/student—for their proper management, these data had to be looked at with an expert eye if they were to be meaningful. There were no strong views about the relative merits of grants or loans for the support of students, but the group was strongly against the notion that undergraduate students, at least, should be salaried and regarded as clients. It was necessary for the university to have strong leadership if it wanted a proper system for setting priorities and wanted to retain proper autonomy.

So, all in all, was their journey really necessary? Most decidedly so, most of the delegates felt at the end. The conference had emphasised once more the strong links which unite European scholarship and the necessity of preserving and nurturing those links.

correspondence

Adding amino acids

SIR,—The issues that Drs Miller and Payne raise (*Nature*, **251**, 176) may be summarised as follows:

1. No example is available of a field trial that shows measurable benefit of amino acid addition;
2. I confuse the problem of meeting physiological needs for protein with that of aesthetic demands;
3. Methionine, not lysine, is limiting in most diets, including cereal diets;
4. Talk of amino acid fortification constitutes a diversion from the main problem, poverty of large masses;
5. The only solution to the problem of malnutrition is to raise the standard of living and improve traditional agriculture.

It is true that examples of completed field trials are lacking. As I stated, (*Nature*, **248**, 643) some are underway; others might well be started in which non-protein calories (such as cassava, sago, and plaintain, or oil) could be added to a predominantly cereal diet with added amino acids to determine how to extend the range of options for obtaining food energy balanced with protein.

I do not confuse physiological needs and aesthetic demands and stated so clearly in the section entitled "No Substitute for Aesthetics".

That methionine is limiting in cereal diets has been disputed. Bressani and Elias[1] pointed out that only when legume contributes more than 50% of the protein in a cereal-legume mixture is methionine limiting; at that level the diet is not protein-deficient.

The world protein food problem has had an extraordinary history: it is the only major world problem that has been discovered and solved, all within a period of about 25 years, and simply by changing standards. More to the point would be statistics on the prevalence of protein–calorie malnutrition of the kind that indicates a particular deficiency of protein—the kind that was called "kwashiorkor". Has this diminished along with the disappearance of the protein problem? And if, indeed, there is an increase of protein–calorie malnutrition of all types, then does this not emphasise even more the need to provide more food energy and point out the consequences of the protein–calorie tradeoff? That amino acid fortification does not reach all elements of a population or that it does not provide additional aesthetics in food is of secondary consequence: the critical point is that additional ways of adding protein make it possible to divert resources to more food energy, or special baby foods, or more pleasing foods.

The world food problem is an extraordinarily complex problem of massive poverty exacerbated by increasing populations and shrinking resources. What Drs Miller and Payne have to offer has been tried again and again, and has been shown inadequate. Only a total world attack can deal with this problem: affluent nations will need to distribute more of their wealth to the poor nations at the cost of a reduction of some aspect of their own quality of life; the poor nations will need to develop policies that reduce the ratio of populations to resources. Ultimately, decisions to act involve moral considerations. But such decisions involve many other factors, among them the availability of solutions and the multiplicity of options. Is it right to deny poor countries the use of technologies that could increase their opportunities to solve problems as they cope with the basic problem of poverty?

AARON M. ALTSCHUL

Washington DC.

[1] Bressani, R., and Elias, L. G., in *New Protein Foods* (edit. by Altschul, A. M.), 282 (Academic Press, 1974).

Publish it not

SIR,—I am profoundly shocked by the leading article in the issue of *Nature* of September 6. My dismay arises from the content of the fourth and fifth paragraphs. Firstly it seems to me to betray a surprising ignorance of the issue under discussion to draw an analogy with weather modification or shocking experimental animals. In both cases we know the consequences to be localised and if a scientist in Idaho does modify the weather, this has no consequences in India or Iceland. Climatic modification is of course a different issue. But with genome fusions, there is no reason to believe that a blunder might not have consequences which would affect very large numbers of people over a very wide area of the globe.

The second issue relates to your concept of "pushing (papers) into the underground". Certainly there are undergrounds: one is concerned with military research and the other industrial research. But it seems reasonably certain (military matters apart) that the people concerned in this particular field are ones who desire public recognition for their work and who would be discouraged very quickly if they saw that their findings would not lead to the glories of a mention in the Citation Index.

Yours faithfully,

D. W. EWER

London.

Family favourites

SIR,—On page 98 and 99 of vol. 251, there are 3 consecutive contributors named Davies.

Statistical analysis shows that the probability of this arising on the basis of chance is infinitesimally small, so it can only be nepotism!

A. G. GORDON

London.

news and views

Digesting DNA and chromatin

ALTHOUGH the basic double-helical structure of DNA has been known for many years its sequence arrangements and spatial organisation in chromatin have remained poorly understood. The discovery of restriction enzymes, which cleave double-stranded DNA at specific target sites, has given a much needed boost to sequence analysis of DNA. Recently some interesting results concerning the organisation of satellite DNAs have been obtained using the restriction enzyme EcoRI, which cleaves DNA at the sequence GAATTC. The size of DNA fragments generated by the enzyme reflects the separation of target sites and so a limited base sequence complexity, as exists in satellite DNAs, would tend to result in infrequent cleavage.

Botchan *et al.* (*Cold Spring Harb. Symp. quant. Biol.*, **38**, 383; 1974) found that mouse satellite DNA could be separated from the rest of the DNA by cleavage with EcoRI. The satellite DNA formed a slow migrating band on electrophoresis whose fragments ranged in molecular weight from 4×10^6 to 20×10^6, indicating that it is composed of inexact repeating units. In contrast, Botchan (in this issue of Nature, page 288) finds that the same enzyme cleaves bovine satellite I DNA into highly repetitious units of 1,400 base pairs. Renaturation kinetics indicate that these units are internally repetitious. He proposes that two stages were involved in the evolution of satellite I DNA. The first stage involved small duplications, indicated by the renaturation rate, to yield the 1,400 base pair unit, and the second stage involved the production of large tandem repeats of this unit. Such analysis of other satellites should give a clearer picture of how satellite DNAs are organised and perhaps confirm this hypothesis. Mouse satellite DNA, if further analysis does not reveal some higher order of repeating units, may be a case where subsequent divergence has obscured the homogeneity of the tandem arrays.

On another level of DNA organisation recent work points to a highly repetitive association of protein with DNA in chromatin. Olins and Olins (*Science*, **183**, 330; 1974) reported linear arrays of chromatin particles, which they termed ν bodies, each about 70 Å in diameter. They calculated that each ν particle could contain at least one, possibly two, molecule of each of the five main histones and a stretch of DNA of molecular weight between 80,000 and 160,000. Sahasrabuddhe and Van Holde (*J. biol. Chem.*, **249**, 152; 1974) isolated micrococcal nuclease resistant particles constituting 50% of the total DNA from calf thymus chromatin with sedimentation coefficients of between 10S and 12S. These particles contained approximately 100 base pairs and had an estimated diameter of about 80 Å. Similarly Clark and Felsenfeld (*Biochemistry* **13**, 3622; 1974) have found that, on digestion with micrococcal nuclease, 50% of the DNA in chromatin remains as protected areas containing roughly 100 base pairs. Hewish and Burgoyne (*Biochem. biophys. Res. Commun.*, **52**, 504; 1973), on the basis of nuclease digestion studies using an endogenous Ca^{2+}–Mg^{2+} dependent endonuclease of rat liver chromatin, have proposed a simple, basic, repeating structure for chromatin. Kornberg (*Science*, **184**, 868; 1974) has elaborated this into a theory of chromatin structure based on a repeating unit containing two each of the main histones (excluding F1 histone) and about 200 base pairs of DNA forming a flexibly jointed chain. Support for this theory now comes from the work of Noll (*Nature*, **251**, 249; 1974) who finds that using both the endogenous Ca^{2+}–Mg^{2+} dependent endonuclease and micrococcal nuclease approximately 85% of rat liver chromatin can be digested into 11S fragments containing about 200 base pairs. Further digestion, however, causes a breakdown of DNA in these fragments. The isolated fragments contain all five major histones and some nonhistone proteins.

Thus evidence is accumulating for a regular spatial arrangement of proteins in chromatin, the proteins protecting stretches of DNA from nuclease attack. Further studies may show a simple relationship between the 200 and 100 base pair fragments, for example a totally protected 100 base pair unit associated with 100 base pairs which are partially proteoted. T. BARRETT

Termination and the juggernaut polymerase

GENE expression seems in general to be controlled at the initiation of transcription. An alternative mechanism operates, however, during the development of some phages. Initially 'early' genes are transcribed by a host (bacterial) polymerase which is halted by termination signals. Subsequently an anti-termination protein is synthesised to allow transcription beyond these signals. The mechanisms of termination and of anti-termination have not been well defined and their role within the uninfected bacterium remains particularly unclear. Adya, Gottesman and De Crombrugghe (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 2534–2538; 1974) now suggest that the mode of termination may be dictated by events taking place at initiation; their work with polar mutants also reveals a new aspect of the connection between translation and transcription.

Expression of the galactose operon of *Escherichia coli* is different when caused by readthrough from a prophage instead of the normal initiation at a galactose promotor. When prophage lambda is induced, constitutive ('escape') synthesis of the nearby galactose enzymes ensues, for two reasons: (1) replication of the galactose structural genes together with the prophage increases the number of operators so that insufficient repressor is present in the cell to prevent transcription; (2) transcription is initiated at the p_1 (*sex*) promotor and proceeds from the prophage, through the intervening bacterial genes, into the galactose operon. Certain mutations permit only the second mechanism to operate, by preventing the replication and the excision from the chromosome of the prophage.

Adya *et al.* used an increase in temperature from 32° C to 41° C to induce prophage bearing such mutations. They found a seven-fold increase in the specific activity of galactokinase, the enzyme coded by *galK*, the last of the three structural genes of the galactose operon. Since the addition of fucose, an inducer of the galactose operon, causes a further increase in galactokinase synthesis, apparently to the same extent as usual, expression by induction and expression by readthrough seem to be additive. This suggests that they take place by different

mechanisms; and implies that galactose repressor can inhibit only transcription that initiates at the adjacent galactose promotor, and does not act simply by blocking progress of polymerase into the structural genes. This is consistent with the observation of Nakanishi *et al.* (*J. biol. Chem.*, **248**, 5937–5942; 1973) that repressor and polymerase seem to compete *in vitro* for galactose DNA and also with the dual control noticed in the galactose operon—there appear to be two operator/promotor sites, whose expression is additive but each of whose repression does not prevent expression from the other (see Hua and Markovitz, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 507–511; 1974).

Termination of transcription in bacterial DNA is not well defined, but sites in λ DNA have been mapped at which rho factor causes RNA polymerase to cease transcription *in vitro*. As the λ map below shows, one site, (t_l), terminates leftward early transcription from p_l; another, (t_r), terminates rightward early transcription from p_r.

Poly (C) in viruses

PORTER, Fellner and Carey (*Nature*, **248**, 675–678; 1974) recently showed that encephalomyocarditis (EMC) virus RNA contains a poly (C) tract which is about 100 nucleotides long and contains 80–90 C residues. The authors argued that the poly (C) tract is probably not translated into protein but instead suggested that it may be involved in viral replication, possibly as a replicase binding site. On page 342 of this issue of *Nature*, Brown, Newman, Stott, Porter, Frisby, Newton, Carey and Fellner report a similar analysis of several other picornaviruses, including some of economic and medical importance, in the hope that an understanding of the universality of poly (C) might give some clue as to its function. In addition, they suggest that if poly (C) should prove to be virus specific then it may be possible to exploit the presence of this unusual structural feature in the design of specific antiviral reagents.

Picornaviruses can be divided by physiochemical methods into five subgroups: enteroviruses (for example polio, swine vesicular disease), cardioviruses (such as EMC, Maus Elberfeld (ME)), foot and mouth disease viruses (FMDV) and human and equine rhinoviruses. Poly (C) tracts were detected in all the cardioviruses and FMD viruses examined but not in six different enteroviruses, three human rhinoviruses, nor in the togavirus Semliki Forest Virus. The presence of poly (C) is therefore genus specific, and Brown *et al.* point out that their oligonucleotide 'fingerprinting' data supports the subgrouping of picornaviruses mentioned above.

The length of the poly (C) tract also varies, not only between viral subgroups but surprisingly between different strains and different subtypes of one serotype. For example the cardioviruses EMC and ME have poly (C) tracts of 100 and 150 nucleotides respectively; and FMDV strains A61 and CGC, 150 and 200 nucleotides respectively.

The location of the poly (C) within the viral RNA molecules is not yet certain but it is possible that the longest fragments originate from the 3′ terminus of the viral RNA. If this proves to be the case then it is not surprising that the enteroviruses do not contain poly (C) since they contain the more familiar poly (A) tail in this position. At present the function of poly (C), like that of poly (A), is unknown; it will be of interest to know whether similar structures are found in other, more exotic viral RNAs, or in host cell mRNA or HnRNA.

ALAN E. SMITH

Since only the early genes *N* and *tof* are expressed upon induction *in vivo* prior to synthesis of N protein, the same mechanism presumably prevails in the cell. It is the synthesis of N protein which allows transcription to continue to the left past t_l and to the right past t_r.

galK galT galE galO/P . . . exo cIII t_l *N* p_l/o_l p_r/o_r *tof* t_r *O P Q*

The accepted interpretation of these results has been to suppose that N protein acts at the sites t_l and t_r where transcription terminates. But another interpretation is supported by the results of Adya *et al.* Readthrough transcription of the galactose genes depends upon N protein since with an N^- prophage there is no escape synthesis of galactokinase—presumably transcription halts at the t_l site in the absence of N protein. That the readthrough transcription supported by N has started at p_l is shown by the reduced readthrough synthesis of galactokinase found when a prophage bearing the *sex* mutation in p_l is induced. The 12 min delay between prophage induction and galactokinase synthesis also is consistent with the idea that RNA polymerase must traverse the long distance between p_l and the *gal* genes; and the RNA made by readthrough is much larger than the usual 12–22S mRNA produced by fucose induction.

Mutants in which foreign DNA has been inserted into the galactose operon constitute one of the few cases in which rho terminates transcription *in vitro*; De Crombugghe *et al.* (*Nature new Biol.*, **241**, 260–264; 1973) suggested that the polar effects of these mutations *in vivo* result from termination caused by rho and not by nonsense codons. Polar mutations inactivate the gene in which they are located but also reduce the extent of expression of subsequent genes in the operon. But when transcribed by N-dependent readthrough from the p_l promotor, the polarity of such an insertion in *galE* is suppressed; galactokinase can be synthesised. Even more surprising is that the polarity of an entirely different type of insertion (not rho-sensitive) and a polar ochre nonsense mutation also are suppressed under readthrough conditions.

The ability of polymerases that initiate transcription at p_l to pass subsequent rho-dependent termination sites thus contrasts with the inability to do so of polymerases that initiate at the galactose promotor. The N protein responsible for anti-termination cannot, therefore, simply act at the termination sites at which its effect is exerted. Rather does anti-termination depend upon where the polymerase initiates transcription. Since phage 21 (which differs in the *N* gene from phage λ) canot complement λN^- mutants to allow readthrough, the site of action of N must be located in the region that is non-homologous between the phages. This would be consistent with the idea that N acts at the p_l site, attaching to polymerase there, and turning it into what Adya *et al.* describe as a "juggernaut" that ignores all rho-dependent signals for termination. Thus only polymerases initiating at the site where N attaches to the enzyme can acquire the juggernaut capacity; all other polymerases terminate normally at rho-dependent sites. The polymerase is seen as an enzyme to which other proteins may attach, in this case conferring anti-termination ability, perhaps in other cases changing the specificity of initiation.

The effect on transcription of the initiation site is also seen by comparing the tryptophan operon located on the *E. coli* chromosome with that translocated to a position to the left of gene *N* of phage λ when readthrough can occur. Segawa and Imamoto (*J. molec. Biol.*, **87**, No. 4; 1974) show that nonsense mutations in the *trpE* (first) gene cause production of truncated messengers when transcription initiates at the tryptophan promotor, but long messengers continue to be synthesised when transcription instead takes place by readthrough from p_l. Results showing that polarity of nonsense mutations is suppressed by readthrough transcription are cited from two other groups as in press

(Zalkin, Yanofsky and Squires, *J. biol. Chem.*; Franklin, *J. molec. Biol.*).

The ability of a juggernaut polymerase to suppress polar nonsense mutants implies that all polar effects may be mediated at the level of transcription. This resolves a conflict of some years standing as to how premature termination of protein synthesis may affect the availability of messenger for subsequent genes. One view has been that dissociation of ribosomes within (but not at the end of) a gene makes the subsequent messenger regions susceptible to degradation; an alternative was to suppose that termination of transcription within a gene causes transcription to halt, although it has always been difficult to visualise molecular models for this relationship (see Lewin, *Nature new Biol.*, **232**, 161–162; 1971). In view of the action of N as an anti-terminator, these new results support the idea that premature translational termination may provoke a rho-dependent transcriptional termination that can be antagonised by the anti-terminator action of N.

Adhya *et al.* propose that termination of translation in a gene allows rho-dependent termination of transcription to take place at the end of the gene by a normal polymerase, although a juggernaut polymerase may of course read straight through, suppressing the polarity of the mutation. This implies that a rho-dependent termination site is present at the end of each gene in which polar mutations may occur but is rarely recognised in the usual course of events. The termination signal at the end of an operon presumably differs from any internal signals and is recognised by all polymerases.

BENJAMIN LEWIN

Do pulsars make supernovae ?

from James Pringle

THE brilliant explosion of a supernova heralds the demise of a highly-evolved, massive star. When stars in the mass range 3–8 solar masses ($M\odot$) have exhausted the hydrogen and helium in their central regions, they all evolve to red-giant configurations of very similar structure. They have a dense core of about $1M\odot$, surrounded by a zone containing shells of burning helium and hydrogen, the whole of which is engulfed in a highly extended low-density envelope about a thousand times larger than the Sun.

The details of what happens next are still uncertain. It is known that some kind of explosion is triggered off, releasing in all about 10^{50} erg. The outer layers of the star are blown off at speeds of some 8,000 km s^{-1}, gathering up the interstellar gas in their path and later becoming the wisps of gas which are known as 'supernova remnants' and of which the Crab Nebula is the most oft quoted example. Eventually, the 'remnant' itself disperses to become part of the ambient interstellar medium which, in its turn, condenses to form stars. It is precisely because a supernova explosion spews heavy elements from the heart of the dead star into the interstellar medium, that planets too can form and life as it is known can exist.

Several specific mechanisms for triggering the initial explosion have been advanced—for example, the suggestions by Fowler and Hoyle that the core collapses caused by the photodisintegration of iron or that the explosive onset of carbon burning disintegrates the whole star. Recent evolutionary calculations strongly indicate that collapse of the core in a highly evolved star, rather than its total disruption, is possible, at least for stars of 3–8 $M\odot$. The collapsed core forms a 'neutron star'—a highly compact object with a typical density of 10^{16} g cm^{-3}. This density is so great that the star's original magnetic field is compressed to a value around 10^{12} G and any rotation the core had before collapse is amplified until the newborn neutron star has a rotation period of a few milliseconds. Thus a sizeable fraction of the gravitational energy released by the collapse of the core is converted into and stored as the rotational energy of the compact object. The combination of rapid rotation and strong magnetic field causes most of the kinetic energy thus stored to be radiated away within a few thousand years, although the slow radiation of the residual rotational energy can be observed for much longer in the radio band as the regular bleeps from a pulsar. These ideas are confirmed by the presence of two very short period, and presumably very young, pulsars in supernova remnants —the nebular remnants around the older pulsars would presumably have dissipated by now.

Thus, the implosion of the stellar core is required, first, to release enough momentum to disperse the outer layers of the star at high velocity and, second, to release enough energy to account for the visual light curve. Light curves have been caculated according to several different theories, but, in general, the first requirement is a problem if the energy is released on too short a timescale. Two recent articles, one by Ostriker and Gunn (*Astrophys. J. Lett.*, **164**, L95; 1971) and the other by Bodenheimer and Ostriker (*Astrophys. J.*, **191**, 465; 1974) suggest that the pulsar at the centre of the supernova can make a substantial contribution to the energy of the outburst. Rather than having to release all the energy in one initial explosion, the authors argue that the power continuously radiated by the newborn pulsar is sufficient to assist in propelling the outer stellar layers up to high velocities. They perform detailed calulations, assuming that the main energy source in a supernova is the dipole radiation from the central pulsar. If it is intense enough, the radiation hollows out a cavity in the centre of the star and then expels the outer envelope at high speed. As this shell of material moves outward, its density decreases rapidly and its transparency increases accordingly. The peak of the light curve occurs when the timescale for the expansion of the envelope is approximately equal to the timescale on which photons can diffuse outwards through it. The authors obtain good agreement with the observations of 'Type II' supernova light curves. They comment on other effects—such as the possible presence of a dust shell around the pre-supernova star—which add complications and could improve agreement. They predict that about 3–4 months after the light curve maximum ultraviolet and X radiation from the central cavity should become visible.

Many a marmot

from our Animal Ecology Correspondent

ALL populations have a tendency to overproduce and, if they are not held in check, to damage the environment upon which they depend for existence. Some ecologists believe that control is enforced only by the actions of extra-population forces, such as the climate. Others hold that mechanisms which are dependent on the density of the population and which modify their effect according to the state of the population are at work. In proposing this system of control, Nicholson stated that the only perfect density-dependent regulatory factor is intraspecific competition; all other factors play less direct, though strongly influential parts (*J. Anim. Ecol.*, **2**, 132; 1933). Wynne-Edwards endorsed Nicholson's views and drew attention to the part played by social behaviour in the dispersion, and hence regulation, of animal populations (*Animal Dispersion in Relation to Social Behaviour*, Oliver and Boyd, Edinburgh, 1962). He stressed that intraspecific competition, be it for a territory, individual range, rank position in a hierarchy or the like, is never directed at the environmental resource in short supply but at a substitute. The winner of a substitute goal gains exclusive rights to some primary environmental resource.

If social behaviour really does play such an important part in the prevention of overpopulation (by allowing only a fixed number of goals to be open to competition) one might expect to

observe changes where the environment embraces both harsh and kind habitats. To investigate this the North American marmot offers as good a study animal as has yet ben found. The species are morphologically very alike and occupy a huge range of habitats from the mountain tops above the tree line (Olympic marmot) through the upper forests to the valleys (yellow-bellied marmot) to the plains at sea level (woodchuck). A most interesting gradation in social behaviour has been reported by Barash which, if the interpretations are correct, shows clearly that the quality of social behaviour is correlated with the harshness of the environment (*Science*, **185**, 415; 1974).

Woodchucks living in fields and meadows at low elevation experience a growing season, when their food is rich and abundant, of about 150 days. They are solitary and aggressive. The male–female association last only as long as the brief courtship and mating themselves. Females breed every year and their offspring disperse before the winter. The yellow-bellied marmot lives at elevations where the growing season may last less than 100 days. It is less solitary than the woodchuck but still maintains home areas. They show a greeting behaviour—a pattern never seen in woodchucks—and greet their neighbours two or three times daily. The Olympic marmot is restricted to the treeless alpine meadows of Olympic National Park, Washington, where the growing season may be as short as 40 days. These are highly gregarious animals which live in well organised colonies of adults, 2-year-olds, yearlings and infants. They do not protect home areas and wander about the colony without fear of attack. Their level of aggression is low and in consequence greet one another about ten times as frequently as their yellow-bellied cousins. Females breed every other year and youngsters do not disperse until they are 2 years old. By the time they reach this age they weigh 70% of the adult weight. In comparison woodchucks (weighing 80% of adult weight) disperse before their first winter.

The important thing for a species is to leave the maximum number of surviving offspring that the habitat will carry. Because of the varying durations of growing seasons encountered by marmots the optimum age for dispersal varies between a few months and 2 years. Barash demonstrates that the environment dictates when dispersal takes place through the effect of growth rate on aggressiveness. If overwinter mortality has been high and the population needs replenishing, the level of aggression is a little lower and a higher proportion of the dispersers remain, and *vice versa*—a very clear example of adaptive social behaviour.

Several factors may complicate Barash's simple interpretation—for example, female Olympic marmots breed once in 2 years while the other species bred annually. The reproductive physiology and feeding ecology of a species play a fundamental part in moulding the shape of the behavioural fine tuning. Barash's work demonstrates beautifully that the barrier between ethology and ecology is more imagined than real.

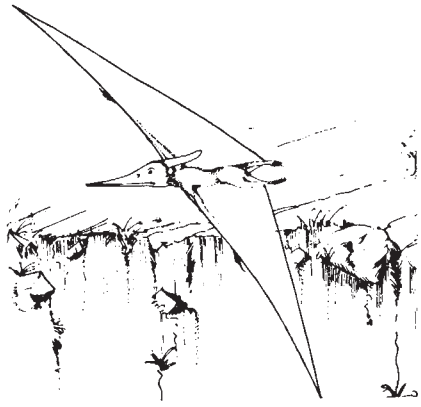
Prehistoric gliders

from a Correspondent

IN a study of the biomechanics of *Pteranodon*, Bramwell and Whitfield discuss the structure and aerodynamics of the largest flying animal to have existed. As *Pteranodon* represented the end product of pterosaur evolution it was likely to show a high degree of structural and aerodynamic refinement and this has been largely confirmed *within this study* (*Phil. Trans. R. Soc.*, **B267**, 503–592; 1974).

Any such study as this is based upon conjecture—indeed herein lies one of its fascinations—but the authors have minimised uncertainty in this respect by utilising an interdisciplinary approach, combining both engineering and palaeontology to formulate conclusions. The application of engineering to an extinct animal is obviously extremely difficult, particularly as parts of the fossil animal were missing. The authors have, however, used the best data available and where their conclusions are open to doubt this in no way infers a criticism of this study, but of the lack of information particularly within the field of low speed aerodynamics.

From available fossil and estimated sizes the strength of the bones in the wing spars has been calculated. The general factor of safety is approximately 5 which is a reasonable value for a flying animal with the assumed flight activities of *Pteranodon*. The most surprising feature of the structure is a factor of safety of only 2 for the humerus. It is possible that the assumed strength values for this bone were incorrect and the authors have considered this as one possibility by suggesting that a stronger, more brittle bone material was practical. They also put forward a more feasible explanation in that part of the loads could be taken by some of the flight muscles being inserted well out along the humerus.



Bramwell and Whitfield's interpretation of how *Pteranodon* soared in the hill lift caused by a cliff.

From tests in a wind tunnel Bramwell and Whitfield provide a new explanation of the large crest on the animal's head. This structure had previously been thought to have the effect of a rudder or steering device but these authors clearly show that it was a weight-saving device; by balancing the aerodynamic loads on the beak it could reduce the required neck muscles and thereby save weight.

The aerodynamic characteristics of the wing were based on the use of data for the Gottingen 417a curved plate as being the nearest to the *Pteranodon* wing section. There is a discrepancy between the drag values of the Gottingen 417a aerofoil section as presented by Schmitz and that presented in Figure 44 by Bramwell and Whitfield, but it is obvious that these authors have used the data from Schmitz for all their performance estimations.

It is unfortunate that there are no more recent data than those for the 417a section nor any more appropriate aerofoil section on which to base the estimations of *Pteranodon*'s performance. From a polar curve of *Pteranodon* the best flying speed was about 8 m s^{-1} with a minimum sinking speed of 0.43 m s^{-1}. The highest useful flying speed was about 14 m s^{-1}. These estimates of performance are realistic as any small discrepancies in the assumed wing drag characteristics are unlikely to have a profound effect on the overall lift/drag ratio.

A comparison between the performances of *Pteranodon*, an albatross and a modern high performance glider shows that *Pteranodon* was a very efficient glider with a sinking speed of about half that of the other two. From estimates of power required it seems that *Pteranodon* was also just capable of powered flight for short durations.

Bramwell and Whitfield conclude that *Pteranodon* was primarily a glider that utilised sea thermals for lift while searching for food. Although dynamic soaring is used by the albatross to sustain flight over the sea, the authors show that *Pteranodon* was unlikely to utilise this form of lift because of its low flying speed and the critical conditions for the required wind gradient.

Heat flow in a metamorphic crust

from Peter J. Smith

IN investigations which attempt to relate the Earth's heat flow to crustal structure and composition, it has been usual to view the crust in terms of homogeneous igneous rocks. Thus, for example, when Roy *et al.* (*Earth planet. Sci. Lett.*, **5**, 1; 1968) demonstrated the correlation betwen geothermal flux and surface heat production, they made it clear that their measurements and analysis applied specifically to intrusive granitic bodies. But as Smithson and Decker (*Earth planet. Sci. Lett.*, **22**, 215; 1974) point out, although crustal models involving homogeneous igneous rocks have the merit of simplicity they may be a poor general representation of the real Earth. Fifty years ago, Clarke and Washington (*Prof. Pap. U.S. geol. Surv.*, **127**; 1924) could plausibly argue that 95% of crustal rocks are igneous; but modern work is more likely to stress the importance, and even the predominance, of metamorphic rocks with characteristic heterogeneities.

Evidence for the modern view is extensive. The W nd River Mountains of Wyoming, for example, are a broad anticlinal uplift with a core of Precambrian rocks largely comprising migmatite. Borehole data suggest that in places such rock is at least 4 km thick, in addition to which denser metasedimentary rocks such as mica schists, garnet schists and metagraywacke may be found at structurally higher levels. Again, a profile through the Ivrea zone of the Alps has been interpreted in terms of an upper crust comprising metamorphic rocks such as mica schist, a middle crust which is granitic in the form of migmatite and granitic gneiss, and a lower crust consisting of dehydrated mafic metamorphic rocks and diorites. Migmatites underlying other metamorphic rock types are also found in Canada, east Greenland, southern Norway and central Europe. Indeed, Barth and Reitan (in *The Precambrian*, Interscience; 1963) have estimated that as much as 93% of the exposed southern Norwegian Precambrian may be metamorphic—a figure which corresponds well with Wilson's picture (in the same volume) of the Canadian Shield as a region of metamorphic rocks "in which areas of granite and other igneous rocks are interspersed".

But where does all this (and much more) leave the analysis of terrestrial heat flow in terms of simple igneous and homogeneous structures? The answer is, of course, a rather poor approximation to the truth, but an approximation which can nevertheless be built on to give a picture closer to reality. Smithson and Decker build by proposing the following three-zone model of stable continental crust:

- The upper zone consists largely of metamorphic rocks of intermediate composition with a mean density in the range 2.75–2.80 g cm^{-3} and seismic P wave velocities ranging from 6.2 to 6.5 km s^{-1}. Geological relationships and gravity interpretations suggest that this surface zone could be 5–12 km thick, although Smithson and Decker use a thickness of 8 km. The metamorphic rocks actually comprise mica schists, metagraywackes (biotite gneiss and quartzo-feldspathic gneiss), greenstones and amphibolites. According to Shaw (*Geochim. cosmochim. Acta*, **31**, 1111; 1961), the mean heat production within the Canadian Shield is 4 heat generation units (h.g.u.); and it is known that intrusive granites generally have a higher heat production. The intervening metamorphic rocks of the upper zone must therefore have a heat production lower than 4 h.g.u. The estimate made by Smithson and Decker is 3 h.g.u., based on a mixture of mica schists, metagraywacke and mafic rocks.
- The middle zone is also 8 km thick and comprises rocks which are less dense and more granitic than those of the zone above. This is the migmatite zone and consists largely of granitic gneiss and augen gneiss intermixed and interlayed with biotite-rich gneiss and amphibolite lenses. The mean density of this very heterogeneous region is about 2.72 g cm^{-3}, P wave velocities lie in the range 6.0–6.3 km s^{-1} and the highly variable heat production has a probable average of about 5 h.g.u.
- The lower zone, 18 km thick, is composed of dense rocks (2.80–3.00 g cm^{-3}) with high seismic velocities (6.5–7.0 km s^{-1}). Both field and laboratory studies show that at likely pressures and temperatures in the lower crust mafic rocks would be recrystallised as metamorphic rocks in the amphibolite, granulite or eclogite facies, although the last of these is unlikely. Smithson and Decker argue that granulite facies rocks actually comprise the bulk of the lower crust, although they expect pockets of amphibolite to coexist with them. Heat production is estimated to be 0.5–.5 h.g.u.

Having proposed this more "realistic" model, however, Smithson and Decker are at pains to emphasise that the zones should not be interpreted as discrete layers. The zones are heterogeneous and grade into each other irregularly, so that even the quoted thicknesses cannot be regarded as universal. Average values tend to conceal considerable diversity in both the horizontal and vertical directions. Thus the model is a generalisation which may apply in the specific form given only in relatively few localities.

Notwithstanding these restrictions, if the surface heat flow for stable continental areas is taken to be 1.2 heat flow units (h.f.u.) in accordance with the suggestion from Blackwell (in *The Structure and Physical Properties of the Earth's Crust*, American Geophysical Union, 1971), the model predicts a heat flow contribution of 0.3–0.5 h.f.u. from the upper mantle. The upper limit of this range then leads to temperatures of 130° C at a depth of 8 km, 407° C for the Moho at 34 km, and 538° C at 54 km in the upper mantle—temperatures which are comparable with, but slightly lower than, those obtained by Blackwell from other models. The controversial point to arise here is the claim by Smithson and Decker that such low temperatures rule out the possibility of a universal seismic low-velocity zone produced in the crust by thermal effects. A low-velocity zone may arise in places in the upper or middle crust because of variations in composition, but it would be unlikely to exist over an area of even continental extent.

In search of a meaning

from a Correspondent

THE first plenary meeting of the International Society for Research on Aggression, whose objects are as much educational as scientific, was held in Toronto on August 17 and 18. The constitution of the society refers to "the destructive and constructive aspects of aggression", but does not define aggression: and in fact definition was the most prominent theme of the meeting, which was attended by fifty or so researchers (about one-fifth of the total membership) from fields as various as anthropology, psychiatry, and experimental psychology.

On the question of definition, K. Lagerspetz (Swedish University, Turku) pointed out that although most definitions include the delivery of noxious stimulation to another organism, this does not always apply in psychiatry or the social sciences. She asked whether, nonetheless, unifying concepts exist for all the fields of behaviour to which the term 'aggressive' is applied. In her view the notion of an 'aggressive drive' is not useful; and is critical of the frustration hypothesis and analyses in terms of learning and inhibitions.

Adopting an historical perspective, J. P. Scott (Bowling Green State University, Ohio) suggested 'disaggregation', or disruption of normal social patterns, as a general concomitant of anti-social violence in both human and animal societies.

The pluralism advocated by Lagerspetz was echoed in a discussion on classification and definition. K. E. Moyer (Carnegie-Mellon University,

Pittsburgh), S. Rosenzweig (Washington University, St Louis) and P. A. Corning (Stanford University) all emphasised the heterogeneity of the phenomena with which members of the society are concerned. The complexities of human behaviour in this regard were exemplified in a remark by J. Paddock (University of the Americas, Oaxaca), during an absorbing communication on anti-violent and 'normal' communities in which he remarked that non-violent and anti-violent attitudes can be militant.

Education as a possible unifying activity for the society was proposed, and briefly discussed. Authentic information on anti-social violence in human communities and on the social and intolerant behaviour of animals, is much needed, notably by teachers in schools and universities; at present they have to contend with a flood of misinformation and entertaining but unscientific writing. The refreshing rigor of the papers and discussions at this meeting suggest that the society could do much to remedy this situation.

Lamb shifts from intensity measurements

from B. P. Kibble

PHYSICISTS at the University of Windsor, Canada have found an interesting new way to measure the small energy change known as a Lamb shift which is the effect of radiation reacting on the atom emitting it. In their report Wijngaarden, Drake and Farago (*Phys. Rev. Lett.*, **33**, 4; 1974), following the theoretical treatment of Drake and Grimley (*Phys. Rev.*, **A8**, 157; 1973), describe an indirect measurement in terms of an intensity ratio of the Lamb shift in the lowest excited states of hydrogen.

Two of the states concerned, denoted as $2P_{1/2}$ and $2P_{3/2}$, have angular momentum associated with them which differs from that associated with the ground state. An atom in either of these states can revert to the ground state by emitting photons of slightly different energies as, because of relativistic corrections, these states have energies differing by a small amount ΔE. A third state denoted as $2S_{1/2}$ also differs in shift $\mathcal{S}$ approximately equal to 0.1 energy from the $2P_{1/2}$ state by a Lamb ΔE, but has associated angular momentum which is the same as that of the ground state so that an atom in it does not radiate a photon. In previous measurements transitions between this S state and the P states resulting in photon emission could be induced by a radiofrequency field whose frequency matches, and is a measure of, the energy differences $\mathcal{S}$ and $\Delta E - \mathcal{S}$.

The alternative approach of the Canadian workers is to note that when the 'pure' states are mixed by the presence of an electric field, the total photon emission is no longer equal in all directions, but is greater in the direction of the electric field than in the perpendicular direction. The ratio is related to $\mathcal{S}/\Delta E$ and since ΔE is already known very precisely from radiofrequency spectroscopy, $\mathcal{S}$ can be deduced.

Measuring radiative intensities, even when only the ratio is required, does not seem likely to be very accurate but there are particularly favourable features in this case. The photons are energetic enough for a high proportion of them to eject countable single electrons from a detector surface and, when increased by an electron multiplier, the resulting pulses of electrons can be accurately counted. If the difficulties of variation of this proportion and of the discriminator level which differentiates between large signal pulses and small noise pulses can be overcome, the direction of the electric field can be controlled sufficiently precisely, the results can be extrapolated to a condition of zero electric field strength and corrected for the occasional coincidence of pulses, then an accuracy comparable to that obtained by radiofrequency methods may be attained. So far Wijngaarden and his colleagues report an accuracy of about 1%, which is two orders of magnitude less than that obtained by radiofrequency methods, but the prospects for improvement are encouraging. The method may well be suitable for Lamb shifts in heavier hydrogen-like ions where a method based on the total photon emission from the S state by application of an electric field has hitherto been used.

Early mammalian development

from Michael Balls and Arthur Wild

RECENT dramatic technical developments have enabled the mammalian embryo, which normally remains hidden within the female reproductive tract, to be viewed and manipulated. These advances were reflected in the programme of the twenty-seventh meeting of the British Society for Developmental Biology, on the early development of mammals, held at the University of East Anglia on September 9–12. In a group of papers on *in vitro* techniques, Dr D. Whittingham (University of Cambridge) dealt with problems related to the fertilisation, culture and storage of mammalian ova, and Dr M. Kaufman (Weizman Institute, Revohot) reviewed recent developments in experimental parthenogenesis and considered possible reasons for the death of parthenogenetic mouse embryos in early post-implantation stages. Dr A. McLaren (University of Edinburgh) discussed the culture of embryos over the period when implantation would normally be occurring *in vivo*, and the culture of post-implantation rat embryos was described by Dr C. Steele (University of Cambridge).

Contrary to Dalcq's rigid predetermination idea, experiments reviewed and described in papers by Dr I. Wilson (University College of North Wales, Bangor) and Dr S. Kelly (University of Oxford) clearly demonstrated the totipotency of the blastomeres up to and including the eight-cell stage, pointing to a labile, epigenetic control of the differentiation of two distinct cell populations (trophoblast and inner cell mass). The further development of these two cell types was considered by Dr R. Gardner (University of Oxford), using chimaeras, and by Dr J. Ansell (University of Cambridge) and Dr M. Sherman (Roche Institute, Nutley, New Jersey), using *in vitro* trophoblast outgrowths.

Abnormalities of development can often be used to throw light on mechanisms operating in normal development; mutations in the complex T locus of the mouse are being studied to this end. Dr N. Hillman (Temple University, Philadelphia) proposed that metabolic errors elicited by mutant *t* alleles are involved in embryonic lethality, whereas Professor D. Bennett (Cornell University, New York) suggested that the T locus controls a specific cell surface antigen which is of great significance in development.

The expression of paternal antigens, especially those of the H-2 locus, could have important immunological conseqeunces in development. One theory as to why the mother does not normally reject the foetus as an allograft is that paternal antigens are not expressed on trophoblast cells, and Dr D. Billington (University of Bristol) discussed the evidence for this hypothesis. Antigen expression was also the subject of a paper by Professor F. Jacob (Institut Pasteur, Paris), but he described experiments on teratocarcinoma cells, which also have great potential for studies on the basis of normal developmental mechanisms. Dr D. Solter (Wistar Institute, Philadelphia) described methods for producing benign teratomas and re-transplantable teratocarcinomas, and the possible immunological factors regulating them. Dr M. Evans (University College, London) dealt with the characteristics of established cell lines derived from teratocarcinomas.

Much is now known about the synthesis of various macromolecules and the fine structural changes that occur in the pre-implantation embryo. The genetic activity underlying these events as expressed in the nuclear and mitochondrial genome was considered by Dr L. Pikó (Pasadena, California). Dr C. Ford (University of Oxford) discussed the effects of genetic imbalance, and a model system for studying its consequences in certain strains of mice; Dr B. Cattanach (Harwell) described sex reversal in mammals and the use of *Sxr* mice in attempts to understand this intriguing problem.

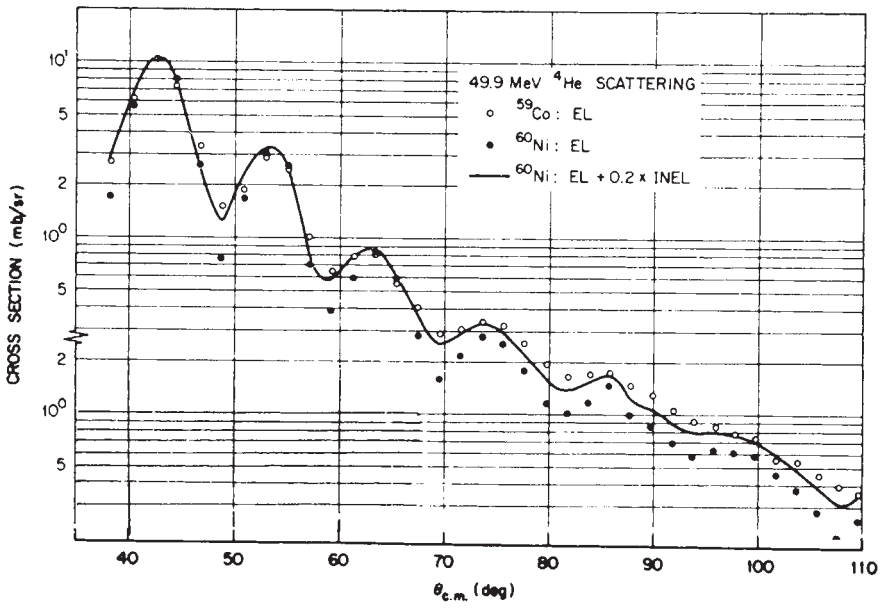
The next group of papers concerned the significance of cell interactions in the morphogenesis of organs, such as the kidney (Dr L. Saxén, University of Helsinki), in pre-natal erythropoiesis (Dr R. Cole, University of Sussex), and in pre-natal lymphopoiesis (Dr M. Ritter, University of Oxford). Dr E. Pantelouris (University of Strathclyde) put forward the hypothesis that the thymus, as evidenced by the effects of genetic athymia, might have an endocrine function in female reproductive physiology, and, finally, Dr J. McKenzie (University of Aberdeen) presented evidence that early cell death might be the underlying cause of human morphological abnormalities hitherto ascribed to so-called 'amniotic bands'. The proceedings of the symposium will be published in the spring or early summer of 1975.

Target-spin effects in elastic scattering

from P. E. Hodgson

MEASUREMENTS of the elastic scattering of helions and alpha particles by nuclei have shown that the differential cross sections for odd nuclei oscillate less strongly with angle than the corresponding cross sections for neighbouring even nuclei. Since even nuclei have spin zero and odd nuclei have spins greater than zero it was at first suggested that this difference may be explained by a coupling between the relative orbital angular momentum **L** and the target spin **I** which can be represented by a **L.I** term in the optical potential. This term is similar to the **L.**σ spin-orbit term which is known to reduce the oscillations of differential cross sections. But this explanation of the difference was rendered unlikely by Satchler's calculations (*Nucl. Phys.*, **45**, 197; 1963) of the magnitude of the **L.I** target-spin term, whch showed that it is far too weak to account for the observed differences in the cross sections.

It has recently been suggested by Satchler and Fulmer (*Phys. Lett.*, **50B**, 309; 1974) that another possible explanation may be found in the different shapes of neighbouring odd and even nuclei due to particle–core coupling. According to this model the odd nucleus is considered as a single particle (or hole) coupled to states of the core with spin **L**, where the core is the adjacent even nucleus. The coupling allows the valence particle to polarise the core and thus gives it a quadrupole moment, and the extra scattering from the quadrupole moment has the effect of smoothing the angular distribution of the scattering from odd nuclei.



Differential cross sections for the elastic scattering of 49.9 MeV α particles by ^{59}Co and by ^{60}Ni showing the difference in the oscillatory structure. The full line is the cross section for ^{59}Co calculated as described in the text from the cross section for ^{60}Ni plus auxiliary data, and agrees well with the ^{59}Co measurements.

To test this explanation, Satchler and Fulmer measured the differential cross section for the elastic scattering of 49.9 MeV α particles by ^{59}Co ($I=7/2$) and by ^{60}Ni ($I=0$). As shown in the figure, the ^{60}Ni cross section oscillates significantly more than the ^{59}Co cross section.

The theory of elastic scattering from odd and even nuclei taking into account the quadrupole moment scattering predicts that they are related by

$$\sigma_{\mathrm{EL}}(\mathrm{odd}) \approx \sigma_{\mathrm{EL}}(\mathrm{even}) + \sum_{L} K_L \sigma_{\mathrm{INEL}} \ (\mathrm{even};\ 0^+ \rightarrow L^+),$$

where K_L is a coefficient that can be found from the measured 2^L-pole moments of the ground state of the odd nucleus and the measured strength of the electromagnetic excitation of the corresponding state in the even nucleus. The inelastic cross section σ_{INEL}(even; $0^+ \rightarrow L^+$) for the excitation of an L^+ state in the even nucleus is also known experimentally. It is thus possible to calculate the elastic scattering from an odd nucleus from measurable properties of the neighbouring even nucleus in a direct way using a simple model.

The ^{59}Co cross section was found in this way from the ^{60}Ni cross section and the appropriate inelastic scattering and electromagnetic transition data, and the results are shown by the full line in the figure. This agrees very well with the measured results for ^{59}Co, indicating that quadrupole moment scattering is indeed responsible for at least the major part of the difference between the scattering from ^{59}Co and ^{60}Ni.

Further calculations showed that the same model is also able to account for the observed differences between the elastic scattering of 60 MeV helions by ^{59}Co and ^{60}Ni. It is likely that it could account for other odd–even differences that have been observed in elastic scattering, except for nuclei with $I=\frac{1}{2}$, for which quadrupole moment scattering is zero. Examples of this occur for ^{89}Y and ^{207}Pb, and such nuclei are therefore suitable for the study of target-spin effects.

This work sheds further light on the character of the optical potential. It would presumably be possible to fit the observed difference between the elastic scattering from odd and even nuclei by including an **L.I** term in the potential and arbitrarily adjusting its strength. This strength would, however, vary from nucleus to nucleus depending on the deformation and would be much greater than the value calculated from the microscopic theory of the optical potential. This is clearly

unsatisfactory, and a much better way of analysing the data is provided by the present work, which shows that a more detailed account of the physics of the interaction enables the data to be understood in a systematic way without arbitrary parameters.

Ins and outs of cell membranes

from a Correspondent

THE chemical assymmetry of biological membranes in the distribution of lipids, proteins and carbohydrates at each face of the lipid bilayer was no doubt implicit in the early membrane models. Recently this proposal has been made explicit, largely through the introduction of a variety of reagents that react to form stable derivatives of membrane components without penetrating the lipid bilayer. The usual experiment is to react in this way intact cells, in which case only components accessible at the outer surface are labelled, or lysed cells when additional sites located at the cytoplasmic side of the surface membrane also become available for reaction.

In a recent issue of the *Journal of Molecular Biology* (**87**, 541–562; 1974) Whiteley and Berg describe two new homologous imidoesters that react under physiological conditions in respectively a non-penetrating or a completely penetrating manner. These reagents can be tagged with ^{3}H or ^{14}C so it is possible to doubly label the membrane in such a way that reactive sites situated at the inner or outer face or both faces of the membrane can be identified. Components that span the membrane can be distinguished from those having copies occupying sites on both sides of the membrane by the sensitivity of the former to proteolysis of intact cells which reduces the size of components accessible on the outer surface.

The non-penetrating reagent described by Whiteley and Berg, (IAI) isethionyl acetimidate ($CH_3.CNH.OCH_2.CH_2.SO_3^-$), is polar and lipid insoluble; the penetrating reagent (EAI), ethyl acetimidate ($CH_3.CNH.OCH_2.CH_3$) is relatively non-polar and lipid soluble. The product of the reaction of these reagents with amino groups is, however, the same amidine. Reaction of intact human erythrocytes or lysed erythrocyte ghosts with EAI labels haemoglobin to the same extent, whereas the haemoglobin of intact cells is only 0.6% as reactive to IAI as free haemoglobin. Therefore, EAI penetrates the membrane and reacts with cytoplasmic proteins at about the same rate as occurs extracellularly in buffer at *p*H 8. The membrane amino groups reacting with these reagents are in proteins or lipids.

When intact cells are labelled with IAI only a fraction (about 2×10^{6} sites per cell) of the amino groups substituted by reaction of the reagent with lysed cells are labelled and 90% of the counts are in protein. There are nearly 20 times as many reactive protein amino groups and nearly 100 times as many lipid amino groups (phosphatidyl serine and phosphatidyl ethanolamine) on the inner membrane surface as on the outer surface of the erythrocyte. These findings are in general agreement with assignments made by other labelling methods.

The use of these new imidoesters however makes an important contribution to recent discussion of the validity of surface labelling techniques. Much of the earlier work has used low concentrations of reagents, and since the labelling often does not proceed to saturation, sites on the inner membrane surface of intact cells may be weakly labelled even by totally penetrating reagents because the large excess of cytoplasmic protein, for example haemoglobin, mops up any reagent appearing at the cytoplasmic face of the surface membrane. Therefore, it is not always completely clear whether the non-reactivity of cytoplasmic face membrane components to a particular reagent means that the reagent is incapable of penetrating the lipid bilayer. This argument has been considered to explain the preferential labelling of outer face components by low concentrations of reagents that were supposed to be completely penetrating. Whiteley and Berg show, however, that the labelling of free membrane amino groups by EAI is the same in intact cells or lysed cells. Labelling of spectrin polypeptides, for example, proceeds to the same extent by exposure of intact cells to EAI or lysed cells (but not intact cells) to IAI (3.0×10^{7} and 1.2×10^{7} amidines per cell respectively). Clearly then, these proteins (which include spectrin and a number of smaller proteins) exist exclusively on the inner surface face and are fully reactive to a completely penetrating reagent added at saturating concentrations. Whiteley and Berg also show convincingly that membrane reorganisation caused by lysis must be slight since intact cells treated with EAI do not incorporate more EAI into membrane components on further reaction with fresh reagent added to lysed cells. It does not, of course, prove that limited reorganisation might not occur during hypotonic lysis, for example, to make certain surface groups more reactive with the non-penetrating imidoester IAI. This can only be decided by mapping the positions of amidine residues along membrane polypeptides labelled under the different conditions.

As an alternative to small chemical reagents, surface labelling with enzymes (lactoperoxidase or galactose oxidase) is extensively used. The reaction of membrane components with galactose oxidase is usually followed by labelling of the aldehyde group generated at C6 of galactose residues by the enzyme with ^{3}H-$NaBH_4$. In a new development with wide practical potential, particularly for the electron microscopic visualisation of membrane glycoproteins, Heitzmann and Richards (*Proc. natn. Acad. Sci., U.S.A.*; in the press) react the aldehyde groups with biotin hydrazide to form hydrozones. The bound biotin is in turn located by ferritin-linked avidin. The association constant of the biotin–avidin interaction is about 10^{15} M^{-1}, that is several orders of magnitude larger than the interaction with a ferritin–lectin, another favourite reagent for localisation of membrane carbohydrate groups. Staining of membranes containing fixed biotin is therefore extremely rapid, minimising non-specific background. If required the galactose oxidase treatment can be dispensed with and a suitable derivative biotinyl-N-hydroxysuccinimide ester is reacted directly with protein amino groups.

The range of enzyme reagents has been extended by the introduction of enzymatic phosphorylation of proteins for the labelling of surface proteins (Kinzel and Mueller, *Biochim. biophys. Acta*, **322**, 337–351; 1973). Protein kinases are ubiquitous enzymes transferring the terminal phosphate of γ-^{32}P-ATP to serine or threonine residues of protein, and usually show a fairly broad substrate specificity. Kinzel and Mueller find that phosphorylation of intact HeLa cells by exogeneously added protein kinase from rat skeletal muscle labels about 15 polypeptide subunits separable by SDS-polyacrylamide gel electrophoresis. A heavily labelled component has an apparent molecular weight of about 55,000. One possible complication using this procedure is the presence of endogenous protein kinases in some cell membranes. The intrinsic phosphorylation of proteins by these kinases probably accounts for the slow appearances of hydroxylamine stable phosphates when cells or isolated plasma membranes are incubated alone with γ-^{32}P-ATP. Phosphoproteins containing aryl phosphate bonds formed during the process of ATP hydrolysis catalysed by surface located ATPases are likely to be less of a problem, particularly if the reaction conditions are arranged so that any inorganic phosphate released from γ-^{32}P-ATP by a surface enzyme is trapped in an external medium containing a high concentration of cold phosphate.

articles

Indian astronomy in the era of Copernicus

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An observatory, in use in India during the time of Copernicus, was part of an astronomical tradition dating back to the second millenium BC.

IN the wake of the quincentenary celebrations for the great Polish astronomer-philosopher Nicolaus Copernicus, it seems appropriate to recall that during his lifetime Indian astronomical methods were being used in Newminster, England as well as in other parts of Europe[1]. At about that time there was also a functional observatory, called *Man Mandir,* in Benares, India, the fame of which attracted European scholars until the nineteenth century.

Scholars and traders went to India from Europe in search of knowledge as well as merchandise much earlier than the sixteenth century, and more steadily after the voyage of Vasco da Gama to Calicut in 1498. During the eighteenth century wealthy patrons and learned societies, such as the Royal Society of London and the Royal Society of Paris, sent scholars to bring back accounts of what they saw.

Among the British scholars visiting the Benares observatory was the distinguished Sir Robert Barker (1729-89) a member of parliament, who gave an eye-witness account:

"I saw a number of instruments yet remaining, in the greatest preservation, stupendously large, immoveable from the spot, and built of stone, some of them being upwards of twenty feet in height; and although they are said to have been erected two hundred years ago, the graduations and divisions on the several arcs appeared as well cut and as accurately divided, as if they had been the performance of a modern artist. The execution in the construction of these instruments exhibited a mathematical exactness in the fixing, bearing and fitting of the several parts in the necessary and sufficient supports to the very large stones that composed them, and in the joining and fastening each into the other means of lead and iron . . . "[2].

Dr Reuben Burrow (1749–92), assistant to Professor Maskelyne the Astronomer Royal at Greenwich, was given the respectable position of Chief Engineer of the East India Company in 1782. In an article entitled, "Hints concerning the observatory of Benares", which is preserved in the British Museum Papers of the Right Honourable Warren Hastings, the first Governor-General of Bengal, from 1774-85 (reprinted in ref. 3). Burrow observed:

"In all the Grecian, Roman and Egyptian remains there does not appear to be the least vestige of an observatory . . . but fortunately for astronomy there is a large quadrant existing at Benares which from the intent of its construction must necessarily have been placed in the plane of the meridian when the observatory was erected; and as this quadrant is an immovable structure of solid massy stones, and consequently not liable to vary its azimuth, or bend like European quadrants, the transits and altitudes of a number of stars may be taken with it, by a proper contrivance; and its position with respect to the meridian and equator and c, found out with the greatest exactness; from whence many useful conclusions may be drawn . . . "[3].

As late as 1823, the writers of the *Encyclopaedia Britannica* considered *Man Mandir* to be one of the five most celebrated observatories of the world, the other four being the Greenwich observatory built by order of Charles II in 1676; the Paris observatory built by order of Louis XIV in 1667; Tycho Brahe's observatory in the Island of Hven, construction of which began in 1576, and the Peking observatory rebuilt by order of the Ch'ien Lung, the Emperor of China in 1744 (ref. 4).

First-hand information available to the Royal Society of London from scholars and experts sent to India led the *Encylopaedia Britannica* to state:

"The science for which the bramins, however, were most remarkable, is that of astronomy; and in this their progress was so great, as even yet to furnish matter of admiration to the moderns."[5]

The interest in astronomical sciences in India, which goes back to the Vedic period, about 1500 BC, was probably cultivated and developed in connection with the religious need for consecrating ceremonies and sacrifices at specific times and seasons. The Vedic rites of Agni-cayana (fire altars) required the construction of shrines of strictly accurate geometrical dimensions where sacred ceremonies were to be performed at a specific and exact time and date[6].

Since the determination of the units of time is contingent upon the accuracy with which the celestial phenomenon can be observed, the needs to study problems of mathematics and astronomy arose simultaneously. This resulted in the determination of solstices and equinoxes, division of the Sun's yearly path into 360 divisions and of the zodiac into twelve equal parts, the discovery of the planets and so on. The pole star was located as the pivot around which the celestial sphere rotated. The Sun was considered the central point, the director of the Earth, around which all the planets revolved. "May the resplendent sun that comes from the centre of the expanse of water of the vast ocean, purify me . . .", and "The sun generates all the earthly directions one by one and controls the seasons. Only the sun is the Lord of our universe."[7].

The growth of astronomical sciences continued with the compilation of the first systematised text of astronomy, *Vedanga Jyotisha,* by Lagadha in about 600 BC (dates vary). This text deals with planetary motions, describes eclipses and divides the ecliptic into 27 or 28 equal parts, called nakshatras. The nakshatras determined the Moon's position in relation to stars each day, the Moon completing its sky journey in 27 or 28 days[8,9].

The acquisition of some of this knowledge during the Vedic period might have been a result of interaction with the Mesopotamian culture, just as there is evidence of the interaction of Greek and Indian Sciences since the early Christian era. It is well known that Greek scholars visited India from time to time, and some of them settled there, contributing towards the development of what may be termed the Indo-Greek Sciences[10]. The concept of Earth being the centre of the Universe was the contribution of the Greeks to the Indian thought.

The *Paulisa Siddhanta,* one of the five treatises on astronomy compiled in Sanskrit by Varaha Mihira in the fifth century AD uses Greek methods of computation, for example, in the *Table of Sines,* the subdivision of the radius is into 120 instead of 60 parts as prevalent in India. This work also mentions the longitude of Alexandria on one hand and of Ujjain and Benares on the other, thus attesting to the significance of Benares (as well as Ujjain) as the centres of astronomical sciences in ancient India[9].

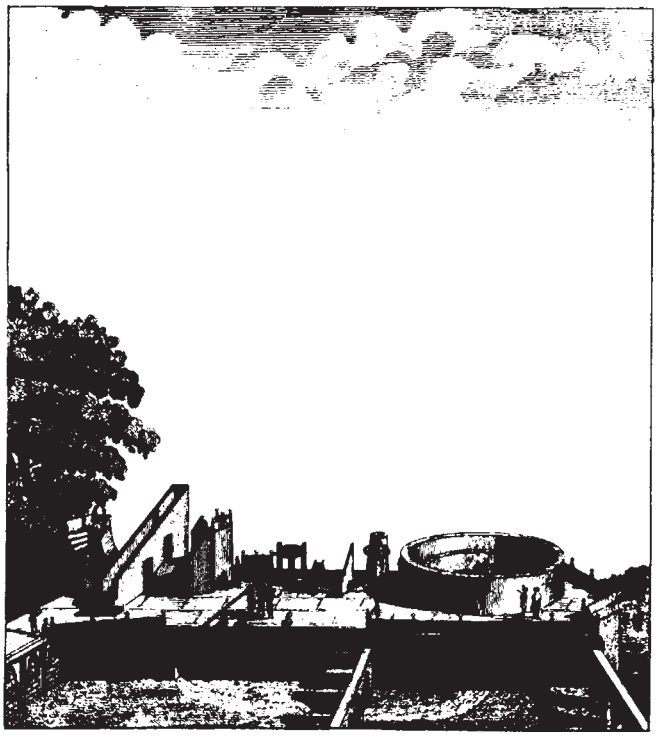

Fig. 1 The observatory at Benares, from the *Encyclopaedia Britannica* of 1778. The drawing is by Sir Robert Campbell, chief engineer of the East India Company.

By the seventh century, the Indian astronomers had discussed astronomical constants and sine tables, explained motions of the Sun, the Moon, and the planets, charted eclipses, and explained the theory of rotation of the Earth. Alberuni, the Persian scholar, who visited India in the eleventh century AD paid a tribute to a seventh century Indian astronomer: "Brahmagupta holds a remarkable place in the history of Eastern civilisation. It was he who taught the Arabs astronomy before they became acquainted with Ptolemy; for the famous Sindhind of Arabian literature frequently mentioned but not yet brought to light, is a translation of his Brahmasiddhanta."[11]. Indian astronomy was introduced in the Middle East after the time of Caliph Al-Mansur (eighth century AD) and subsequently reached Europe. Until the twelfth century AD, the two well known centres of astronomical research were Ujjain and Benares. Ujjain had the same reputation in ancient India as Greenwich has in the modern world. There is, however, no record of astronomical instruments at Ujjain earlier than those installed by Jai Singh in 1734, and even these have disappeared today.

Ancient records were lost because the astronomical sciences, along with the others, were dealt a severe blow after the invasions of Shihabuddin Muhammad Ghouri and his General Qutubuddin Aibak in the latter part of the twelfth century AD. In 1194 Benares was ransacked and almost every monument of ancient Indian civilisation was irretrievably damaged. The annihilation was so complete that no records of ancient learning were spared from destruction. In 1199 Vihara (Bihar), a centre of learning and famous as the site of a college, was laid in ruins. The great university of Nalanda was destroyed, its spacious library burnt to ashes, and practically all Buddhist literature and monks exterminated. The religious fanaticism that continued in the thirteenth and the fourteenth centuries resulted in the collapse of astronomical activity during this period, the last celebrated Indian astronomer being Bhaskara II, 1150 AD. This historical background is essential to an evaluation of the contradictory information now available about the Benares observatory.

Astronomical activity was revived at the beginning of the Copernican era. Works of mathematical astronomy and astronomical calculations, though limited to computation of data and tables and commentary of previous works, were compiled by Gangadhara (1420), Ganakananda (1447) and Makaranda (1478); followed by further works in the sixteenth century by Jnanaraja (1503), Ganesa (1507) and Vallala (1510)[12]. European traders and scholars visiting India in the sixteenth and seventeenth centuries witnessed this last flicker of astronomical work. It was this information that attracted eminent European scholars in the eighteenth century.

The axe of fanaticism again fell in 1669 when the last of the Great Moguls, Aurangzeb Alamgir, sacked Benares "to demolish all the schools and temples of the infidels and to put down their religious teaching and practice".

The origins of Benares observatory therefore are shrouded in obscurity. It is known that Vallala moved to Benares for astronomical studies in about 1500 AD, which might account for the existence of an observatory there. Sir Robert Barker was told in 1772 that the observatory was constructed on the orders of Emperor Akbar in the late sixteenth century. A. Campbell and T. D. Pearse, who visited the observatory in the 1770s stated that the observatory was built two centuries previously by Raja Man Singh son of Jai Singh, and named *Man Mandir* after him. J. L. Williams and William Hunter, who visited India in 1793 and 1798 respectively, on the basis of a newly discovered document, *zeej Muhammadshahi*, the authenticity of which is questionable, considered that the observatory was built in 1743 "for the repose of holy men and pilgrims . . . never used, nor capable of being used for any nice observations . . . (that it was) built more for ostentation than the promotion of useful knowledge.". G. R. Kaye (whose observations are considered incorrect by some authorities) in "A guide to the old observatories", published in 1920, concurred with the opinions of Williams and Hunter, surmising that the Benares observatory was one of the five Jantar Mantars (observatory) erected by Raja Jai Singh of Amber in 1737, the four others being built at Delhi, Ajmer, Ujjain, and Mathura[12]. Prinsep and Tavernier who visited India in the seventeenth century, during the lifetime of another Raja, Jai Singh, considered that he had built the observatory[4].

All the above sources—those who believe that the observatory was built in the sixteenth or the seventeenth century or those who believe that the observatory was built in the eighteenth century—nevertheless agree that it was constructed on the roof of an old building. It is hard to conjecture why anybody would wish to construct new observatory on the roof of an old building, or why that particular building was chosen for the placement of astronomical instruments. It is obvious that Emperor Akbar, Raja Man Singh or Raja Jai Singh (whoever he might have been, as there were several of them), were interested in restoring a historical observatory rather than building a new one. If they wanted to build a new observatory, and that particular building in Benares had no value, they would have done so, as they certainly did not lack the financial resources, closer to their capitals, rather than selecting an old damaged building, that too in Benares. The above considerations including the fact that there has been a tradition of continued astronomical research in India going back to the ancient Vedic period leads to the conclusion that there was an observatory in Benares which was damaged during Shihabuddin's invasion of 1194. Whatever remained of the observatory was of interest to Indian astronomers who carried on limited observations there, and were seeking a patron to restore and renovate it. When political normality returned in the sixteenth century, the old observatory was

restored. If the stories of Jai Chands are true, this again underlines the fact that the observatory was damaged and rebuilt several times, which attests to the historical significance of Benares as an ancient city of astronomical learning. John Call, FRS, while looking at the signs of the zodiac on an ancient monument, observed: "These buildings and temples were places of residence and worship of the original Bramins, and bear the mark of great antiquity, having perhaps been built before the Persian conquest," or, in the words of Barker, "it serves to strengthen the argument of supposing that the Bramins had a knowledge of astronomy before the introduction of Mahometanism into Hindostan (India)[2].

The meaning of *Man Mandir* is "Temple of the Mind," a more appropriate name for this ancient observatory. This old observatory is purely of historic interest, as the methods of observation and the remaining instruments are of no value today. Observations in the past were made with the naked eye using suitable devices for measuring the angles. The telescope was unknown to Indian astronomers. In spite of these limitations, some remarkable calculations were made in ancient India that led to the establishment of an astronomical epoch. The Indian astronomers established an epoch when the Sun, the Moon and all the planets were last present in zero longitude: that was in 3102 BC. The period from one such epoch to the next, according to Aryabhata (fifth century AD) is 1,080,000 yr and is called a Yuga. Four Yugas, the Krtayuga, Tretayuga, Dvaparayuga, and Kaliyuga make a Mahayuga; and 1,008 yugas make a kalpa. The current quarter is the Kaliyuga, assumed to have begun at sunrise at lanka (a hypothetical place where the meridians of Ujjain intersect the equator) on Friday, February 18, 3102 BC. The epoch of zero longitude is apparently useful in computing the mean longitude of a planet[13].

$$\text{mean longitude} = (R \times A)/C$$

where R is the number of revolutions of the planets in a yuga; C is the number of days in a yuga, and A is ahargana, that is the number of days elapsed since the epoch. It is interesting to recall that the Newminster manuscript stated that the era of the flood was 3102 BC, the beginning of the kaliyuga era[14].

It was this type of information that impressed Professor John Playfair (1748-1819) to observe: "Undoubtedly the ancient astronomers must have possessed the best instruments probably differing from modern ones, but fully as powerful."[4]. The writers of the *Encyclopaedia Britannica* commented that the establishment of an astronomical epoch by the Hindoos (same as Hindus, Bramins, Indians) and the observation of this rare phenomenon—the conjunction of Sun, Moon and all the planets according to their mean positions—was a remarkable finding that had received respectable sanction by the French Academy of Sciences[6].

It is also curious that the date of Copernicus' birth, February 19, coincides with the date of the establishment of the astronomical epoch by the Hindus (Bramins), February 18, 3102 BC.

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Rotation of the Italian Peninsula

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Palaeomagnetic measurements of Upper Cretaceous–Palaeogene limestones from the northern Apennines indicate that the entire crust of the Italian Peninsula has rotated anticlockwise through 43°. This is the first direct evidence from Italy of rotation, which is of a comparable magnitude to rotations observed elsewhere in western Europe.

ANTICLOCKWISE rotations of the Iberian peninsula[1], Sardinia[2], Corsica[3], and the western[4] and southern[5-7] Alps have been inferred from palaeomagnetic studies of Permian to mid Tertiary rocks and from geological evidence[8]. An anticlockwise rotation of the Italian peninsula has also been suggested. The rotation of Italy fits some theoretical schemes of Mediterranean palaeogeography. These include originally continuous, linear, E–W mountain belts[9] and microplates packed into the western apex of the Tethys embayment of Pangea[10]. Evidence of rotation has been found in palaeocurrent directions in the Tertiary Apennine flysch[11], and from palaeomagnetic measurements in the southern Alps (Permian and Triassic)[5,6] and the southern Alpine foothills (Tertiary)[12].

Palaeomagnetic studies in the southern Alpine region cannot, however, be used as evidence for rotation of the Italian peninsula, because the two areas are separated by the Po Basin in which the base of the Pliocene is as much as 6,000 m below sea level[13] (twice the present depth of the western Mediterranean basins). These sediments have been strongly folded and faulted, indicating severe tectonic activity. Nothing is known of the pre-Miocene tectonic history of the Po basin; thus it cannot be assumed that evidence from the southern Alps is applicable to the Italian peninsula.

No previous palaeomagnetic studies have been carried out in the Italian peninsula. A major difficulty is that the western part of the peninsula is predominantly covered by allochthonous sequences, and the autochthonous (?) eastern sequences contain no strongly magnetic rocks. We here describe the results of palaeomagnetic studies on a weakly magnetic Upper Cretaceous–Palaeogene limestone formation in the autochthonous or

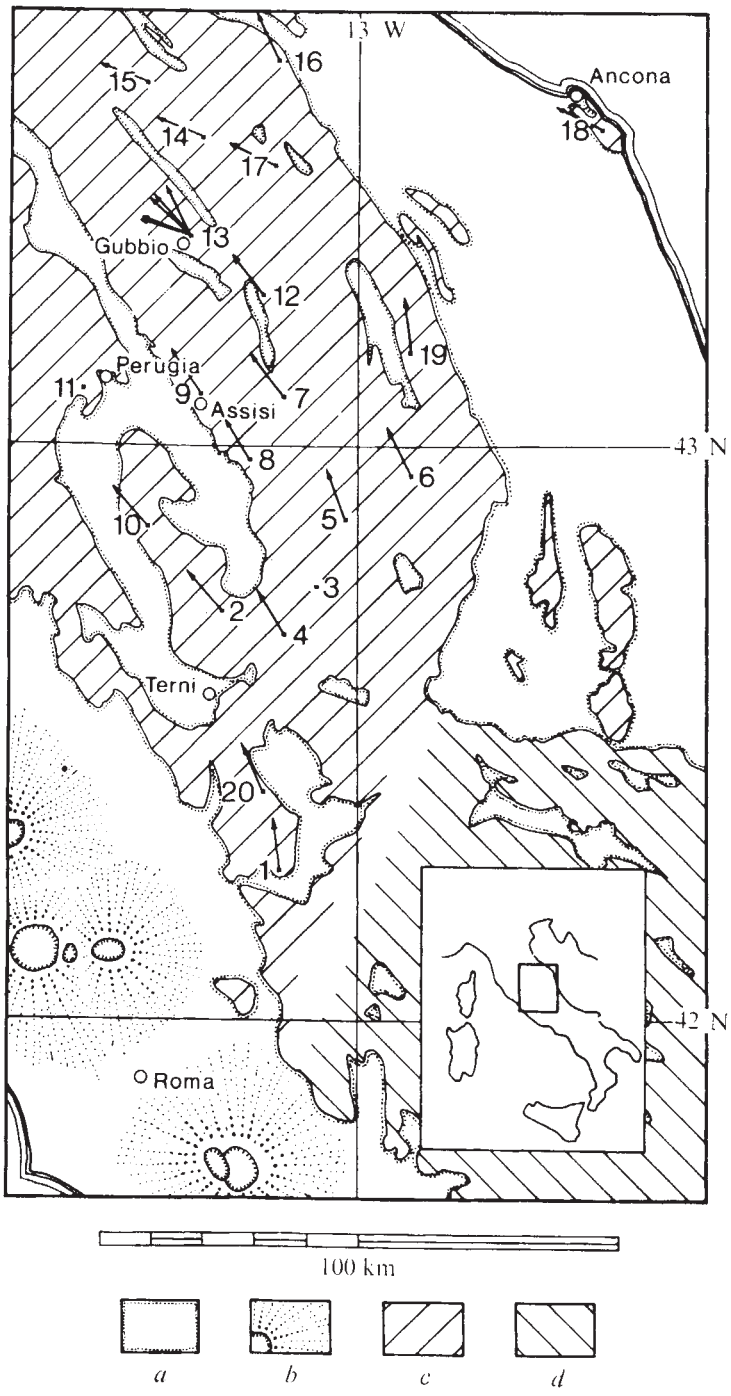

Fig. 1 Location of sample sites; arrows show declination of remanent magnetisation. *a*, Upper Tertiary–Quaternary sedimentary cover; *b*, Quaternary volcanic centres; *c*, Umbrian sequence (Mesozoic–Lower Tertiary); *d*, Abruzzi sequence (Mesozoic–Lower Tertiary).

para-autochthonous Umbrian sequence of the northern Apennines.

A neritic carbonate platform which occupied the area of the miogeosynclinal Umbrian sequence during the early Liassic subsequently broke up into a complex of seamounts and basins and underwent pelagic sedimentation during the remainder of the Jurassic and Cretaceous[14]. Increasingly terrigenous sedimentation in the Palaeogene led to the deposition of Miocene turbidite flysch, and to tectonic deformation in the late Miocene, the Pliocene and the Quaternary[15]. A long interval (Upper Cretaceous–Middle Eocene) is represented by the Scaglia Rossa, a pelagic limestone. Frequent outcrops and quarries expose this attractive stone. The time interval spanned by the formation, and its age and probably autochthonous character make it suitable for palaeomagnetic sampling.

Twenty different localities were sampled (Fig. 1). At locality 13 (the classic profile of the Bottaccione gorge at Gubbio[16,17]) the entire section is exposed, and we placed five sites here at different stratigraphic levels in order to measure the variation in palaeomagnetic directions during the depositional interval. Twenty-four sites were sampled. At each site, 7 to 14 oriented cores were drilled—a total of 180 cores, each of which gave two or three one-inch cylindrical specimens. The remanent magnetisations were very weak—10^{-6} to 10^{-7} gauss—but could be measured with very good repeatability. Susceptibilities were also very low—10^{-6} to 10^{-5} gauss oersted^{-1}.

A representative specimen was selected from each site and subjected to progressive alternating field (AF) demagnetisation in peak fields of up to 400 oersted in intensity. The demagnetisation characteristics were uniform for all the specimens (Fig. 2). After initial removal of an unstable component of magnetisation, the remanent intensity and direction were quite stable throughout the remainder of the demagnetising range, occasionally acquiring an anhysteretic component in 400 oersted. Computer analysis of the demagnetisation curves, utilising one index for directional stability and a separate index for overall magnetisation stability[18] enabled us to select the optimum demagnetisation field for each site. As the optimum field was always less than 200 oersted, all of the specimens were demagnetised in fields of 100 oersted and 200 oersted. Statistical analysis of the remanent directions for each site at each demagnetisation stage gave within-site circles of confidence with an average radius (α_{95}) of 10.7°.

The palaeomagnetic data were satisfactory at all but two of the sites. Local structural and stratigraphical complications obscured the significance of the data from site 3. At site 11, half of the sample directions were closely grouped near the mean normal directions of the other sites, but the other half had normal declinations and negative inclinations. It is possible that a partial field reversal was recorded in these samples. Alternatively, some of the samples may have been accidentally inverted. Because of these ambiguities the data from sites 3 and 11 were excluded from this preliminary analysis.

At site 13A a field reversal was apparently recorded. Most of the samples had normal directions; the remainder were exactly reversed in both inclination and declination. The normal equivalent directions were used in computing the site mean and other statistics.

Evidence from the five sites within locality 13 shows a progressive change in direction. The older sites, lower in the section, have more westerly declinations (by about 35°) than the younger sites. If supported by more detailed study, this implies that some rotation occurred during the mid Cretaceous–mid Eocene. Palaeontological dating of individual sites is at present available in only a few instances. These are in agreement with the established stratigraphy. Further palaeontological work is in progress and hopefully will enable us to refine the present picture.

The pink colour of the limestone at first suggested that secondary components of magnetisation resulting from haematisation may be important. Several tests indicated that this was not the case.

At site 9, samples were taken from adjacent pink and white sections of the same bed. Both sets of samples gave good quality data, similar in intensity, and with well grouped, reversed directions of magnetisation that did not differ significantly from each other. From this we conclude that the pink pigment does not provide an important contribution to the total remanence of the Scaglia Rossa. Thermal demagnetisation of pilot specimens from each site showed a notable decrease in the intensity and increased dispersion of the directions of weak remanence in the temperature range 550–600° C. These results suggest that the dominant magnetic mineral is probably magnetite, although the presence of a haematite phase cannot be completely discounted. Precise determination of the magnetic mineralogy is complicated

by the difficulty of separating the magnetic minerals. Preliminary results indicate, however, that stable, secondary chemical remanent magnetisation is unlikely to be a serious factor affecting the remanent directions of the Scaglia Rossa.

The site mean directions of the remanence after alternating field demagnetisation in 200 oersted are shown in Fig. 3; the reversed directions were used in statistical analysis and in the calculation of the mean direction. The distribution of azimuths was uniform about the mean direction, and the distribution of angular differences from the mean direction was Fisherian at the 90% confidence level. The mean declination and inclination of the Scaglia Rossa were 320.1° and 45.4°, respectively, with $\alpha_{95}=5.9°$.

The mean locations of the late Cretaceous and early Tertiary poles for stable western Europe and the Russian platform are 83°N 194°E, and 75°N 161°E, respectively[19]; the mean of these poles is at 79°N 178°E. The correspond. g direction of site declinations are shown in Fig. 1. All but two of the sites were normally magnetised; the normal equivalents of the

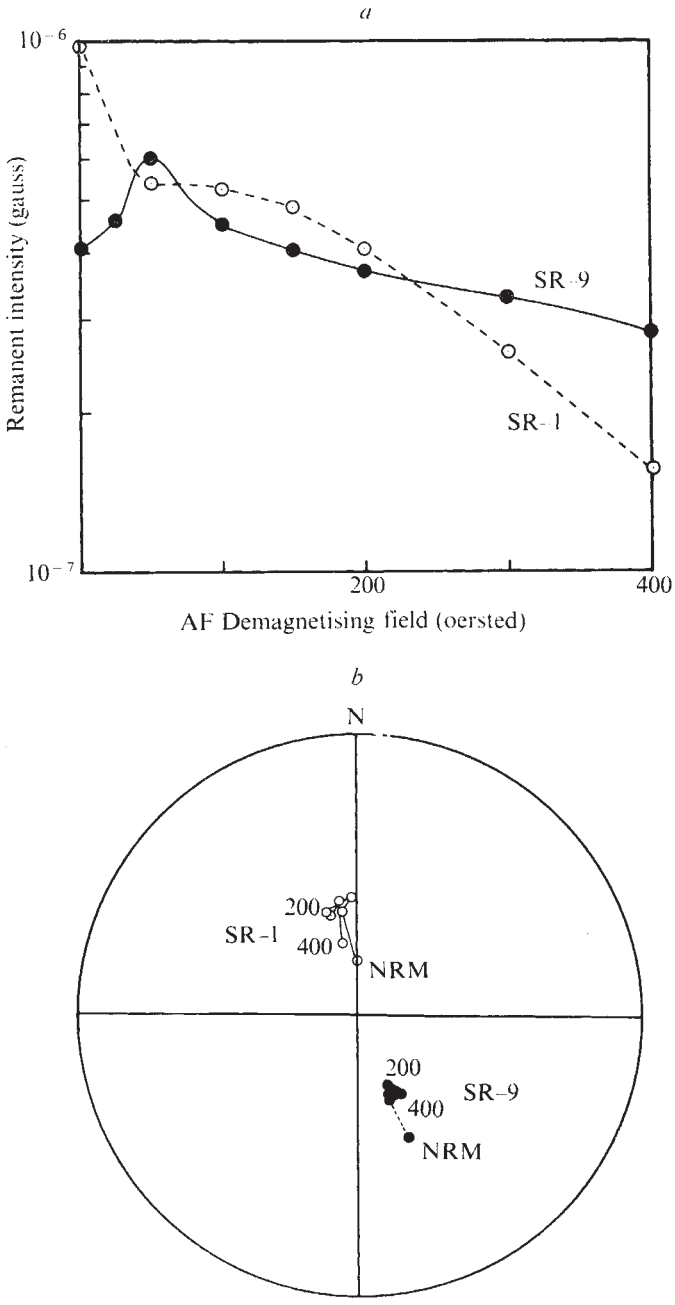

Fig. 2 Intensity (*a*) and direction (*b*) variations of normal (SR-1) and reversed (SR-9) specimens during alternating field (AF) demagnetisation. NRM, Natural remanent magnetisation.

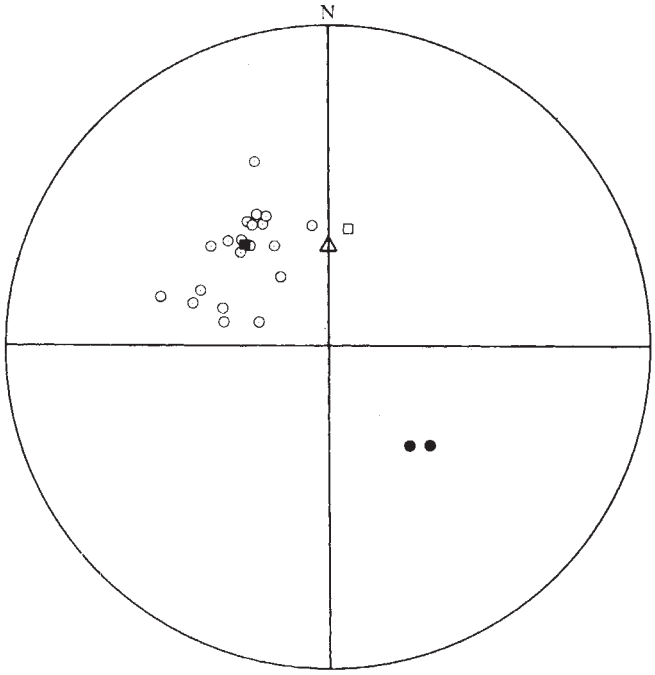

Fig. 3 Stereogram showing site mean directions of remanent magnetisation of the Scaglia Rossa after AF demagnetisation in 200 oersted. Open (solid) circles, normal (reversed) directions; open square and triangle, directions of the early Tertiary and Cretaceous (respectively) geomagnetic fields derived from the appropriate mean pole positions for stable Europe; solid square, mean direction for the Scaglia Rossa.

magnetisation at the centre of our sampling area would have a declination of 4.5° and an inclination of +52°.

That direction differs by 6° in inclination and 43° in declination from the mean direction of our data. Thus, the inclinations are not significantly different and we cannot determine a palaeolatitude change with respect to stable Europe. There is, however, a significant declination difference, which is uniform at all but a few of the most northerly sites (Fig. 1).

The Scaglia Rossa carries a remanence which is probably not affected to any serious extent by secondary magnetisation. The relatively slow pelagic deposition, the long time interval represented by the formation, and the magnitude of the declination difference, show that this difference cannot be a result of secular variation. We therefore conclude that the rocks of the Scaglia Rossa have been physically rotated about a sub-vertical axis.

We envisage three ways in which such rotation could occur. First, there could have been rotation of the Mesozoic–Cainozoic sedimentary cover as a whole, relative to the underlying crust, if the cover is allochthonous above a major *decollement* surface. This, however, would require a relative displacement of about 100 km between the northern and southern parts of the studied area; nowhere have horizontal displacements of more than a few kilometres been observed. Second, the same rotational effect could be produced by simultaneous, independent, *en echelon* rotations of a series of small, allochthonous structures. Although we cannot dismiss this possibility, there is no evidence for it and this tectonic style has never been suggested for the Umbrian Apennines. Third, the entire crust of the Italian Peninsula may have rotated. This interpretation seems to be more compatible with the geological evidence than either of the others.

The 43° counterclockwise rotation is in the same sense as, and is of comparable magnitude to, rotations observed in other parts of the western Mediterranean. This is the first direct evidence that the Italian Peninsula fits this pattern.

A more detailed understanding of the tectonic significance of the rotation will emerge from additional palaeontological and palaeomagnetic sampling and measurement. It should be

possible to determine whether deposition of the Scaglia Rossa occurred during the rotation, as suggested by the palaeomagnetic data from the five sites at locality 13. It should also be possible to test the hypothesis of oroclinal bending as the origin of curvature of the northern Apennines. These studies are still in progress and a complete report will be presented later.

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Bovine satellite I DNA consists of repetitive units 1,400 base pairs in length

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Bovine satellite I DNA was shown by its susceptibility to cleavage by restriction endonucleases to consist of direct tandem repeats 1,400 base pairs in length. Renaturation kinetics indicate that this repeat is itself internally repetitious. These data argue in favour of a DNA replication mechanism for the evolution of this satellite.

THROUGH the light microscope, mitotic chromosomes look highly organised. This organisation is revealed by means of the various cytochemical techniques that show characteristic banding patterns for each metaphase chromosome[1]. Although the molecular basis of these bands is still unknown, it seems clear[2,4] that the local variations in staining intensity seen with a particular dye are a consequence of differences in packing density of the unit chromosomal fibre. Regions selectively stained darker by Giemsa are shown to have a higher concentration of packed DNA than other chromosomal regions when measured by phase contrast microscopy[3,4] and by Feulgen staining[4,5]. In many organisms, the regions adjacent to the centromeres of the chromosomes can be easily identified by these techniques. Several authors have suggested that the dense packing of the chromosomal DNA in this area is due to the regularity of the DNA itself[4,6,7]. The long order organisation of these units is therefore of interest.

Wherever tested, the DNA of this centromeric heterochromatin[8,9] is largely composed of sequences of nucleotides repeated many times. Because these sequences are simple and tandemly arranged, they can often be separated as 'satellites' from the bulk of the DNA by a variety of equilibrium centrifugation techniques. Two approaches have been taken to study the organisation of satellite DNA. First, using renaturation kinetics, Waring and Britten[10] found that mouse satellite renatured with a $C_0t\ \frac{1}{2}$ of 7×10^{-4} mol l^{-1} s. This renaturation data indicated a statistical complexity of 200–300 base pairs for the mouse satellite DNA, and similar data have been obtained for other satellite DNAs. The second experimental approach to satellite organisation has been through various sequencing techniques. The pyrimidine tract analysis of Southern[11] indicated that the guinea pig satellite α, contained a large amount of a repeating hexanucleotide. Superficially, these results seem to be at odds with the observed kinetics of renaturation of satellite α DNA. But Southern's data also showed that only 30% of the guinea pig α satellite was composed of the hexanucleotide and that the remaining sequences were considerably more complex. It also seemed likely that the bulk of the DNA in this satellite was related to the basic unit by simple mutations and rearrangements. Further studies indicate a similar situation in the mouse[12] and kangaroo rat[13] satellites. The most complete sequencing data exist for the *Drosophila virilis* group satellites. Gall and Atherton[14] have found that more than 93% of this satellite is of one heptamer. A resolution between the two approaches discussed has not been reached, although it seems clear that the renaturation complexity cannot be taken as an actual repeat length.

The data from all of these studies do not bear on the existence of a long range order to repeats within satellites. Consider a tandem array where the repeat unit is (*baaaaaa . . . . b*) where both *b* and *a* represent a small sequence. The renaturation rate of such a DNA would be dominated by *a* and would not indicate a higher order repeat. Sequencing experiments without long overlapping fragments from this array might also leave this large repeat undetected. This problem can potentially be resolved by use of restriction enzymes, which cleave double-stranded DNA at specific sequences. If each repeat unit contains a single restriction sequence in it, the whole array of repeats will be cut to the length of one unit. If the sequences of type *b* contain a restriction sequence, but those of type *a* do not, long fragments will be generated which reflect the separation between *b*s, that is the long order repeat. If the site occurs more than once, fragments will be generated, the molecular weights of which will add up to the repeat length.

Britten and Smith[15] have determined that 10% of bovine DNA consists of "highly repetitive" sequences; furthermore, this genome contains four distinct classes of satellite DNA[16,17]. Kurnit *et al.*[16] have found that the four satellites are located on the autosomal chromosomes and in the region of centromeric heterochromatin. The major satellite[16–19], which has been

called satellite I, has been estimated to be 6–7% of the total DNA[16,17]. Here I report studies with restriction endonucleases which show that this satellite DNA has a repeat unit which is 1,400 (± 50) bases long. This higher order repeat unit which accounts for more than 95% of the satellite is internally repetitious.

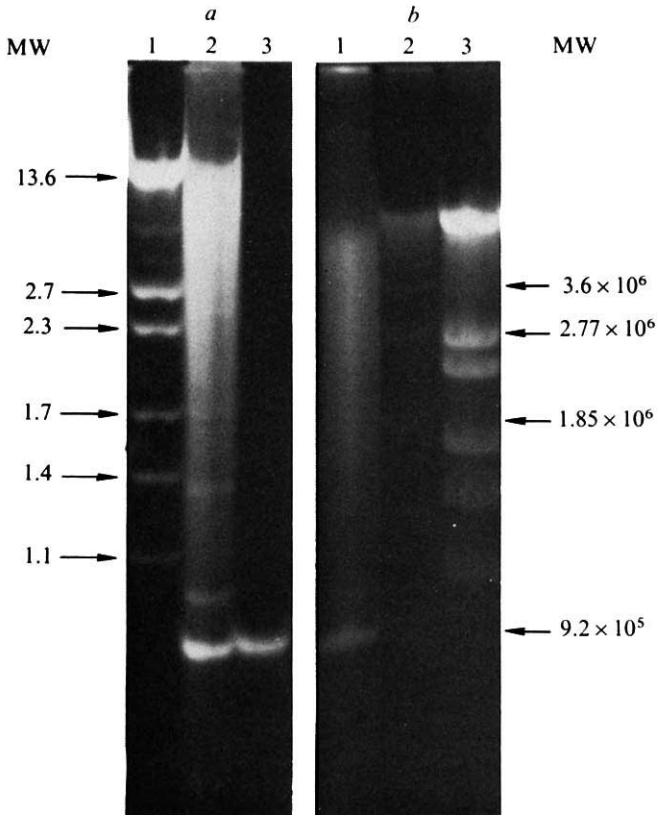

Fig. 1 *a*, Endonuclease EcoRI cleavage of total and satellite I bovine DNA. Bovine kidney cells (MDBK) were grown in Dulbecco's media containing 10% calf serum, and 5 μCi ml^{-1} of ^{32}P as orthophosphate. Confluent monolayers of cells were collected and the DNA extracted as described previously[35]. Cells were lysed in 0.5% SDS and subjected to a pronase (1 mg ml^{-1}) digestion for 8 h in 0.01 M Tris *p*H 7.9 + 0.001 M EDTA. The lysate was extracted with phenol and chloroform-isoamyl-alcohol (24:1 v/v) and then dialysed extensively. After digestion with RNase (100 μg ml^{-1}) and redigestion with pronase, the DNA was re-extracted with phenol. The DNA was then dialysed into 0.01 M Tris *p*H 7.9 + 0.001 M EDTA. The DNA extracted by this procedure had a specific activity of 19,700 c.p.m. μg^{-1}. 100 μg of total DNA was placed in a 5 ml solution of CsCl (density 1.700 g ml^{-1}) and centrifuged for 48 h at 50,000 r.p.m. and then 20 h at 40,000 r.p.m. Fractions corresponding to satellite I DNA were pooled and dialysed into 0.005 M $Na_2B_4O_7$ + 0.005 M Na_2SO_4 *p*H 8.9. $AgNO_3$ was added to an f of 0.40 and the solution brought to a density of 1.500 g ml^{-1} with Cs_2SO_4. The satellite DNA was again banded in this solution by centrifugation as described above. The fractions of the major peak (corresponding to 80% of the DNA in the gradient) were pooled. (1) 2 μg of Ad2 virus DNA, (2) 2 μg total bovine DNA, (3) 1 μg satellite I DNA were digested separately for 2 h in 0.01 M Tris *p*H 7.9 + 0.01 M $MgCl_2$ at 37° C, with EcoRI. The volume of the reaction mixture was 100 ml and contained 0.03 mg ml^{-1} of the restriction enzyme. The enzyme was isolated as described[21,22,36]. The reaction mixtures were adjusted to 20% sucrose and layered onto a 1.5% agarose slab gel. After electrophoresis in E buffer[22,36] at 5 V cm^{-1} for 10 h, the DNA in the gel was stained with ethidium bromide (0.5 μg ml^{-1}). The gel was photographed with ultraviolet excitation of ethidium fluorescence[36]. The molecular weights correspond to the Ad2 fragments A–F shown in (1)[22]. *b*, Partial digestion of satellite I DNA with EcoRI. 1 μg of high molecular weight satellite I DNA was digested with EcoRI as described in Fig. 1*a*, but the reaction was stopped after 20 min by adjusting the mixture to 0.01 M EDTA and 0.5% SDS. The reaction mixture was then subjected to electrophoresis as before. The partial digestion is in position (2). (1) is the total bovine DNA digested to completion and (3) is the Ad2 markers. The molecular weights shown refer to the partial cleavage products.

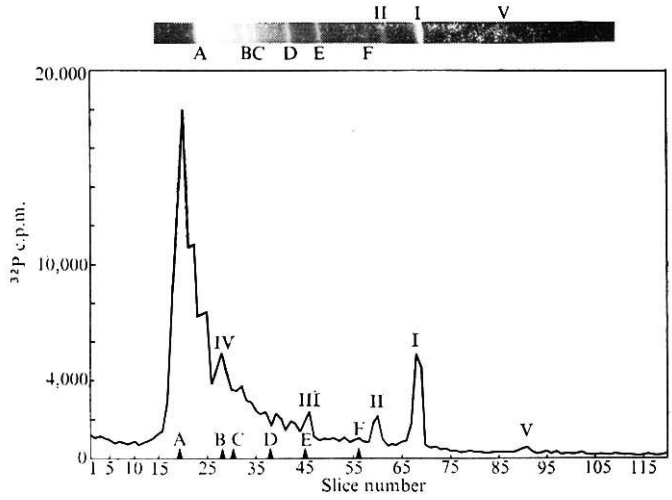

Fig. 2 Distribution of fragment sizes of total bovine DNA after digestion with EcoRI. 7.5 μg of ^{32}P-labelled bovine DNA (specific activity 1.9 × 10^4 c.p.m. μg^{-1}) + 0.3 μg ^3H Ad2 DNA (specific activity 10^6 μg^{-1}) were digested with EcoRI as described in Fig. 1. The reaction was stopped with 0.5% SDS + 0.01 M EDTA and extracted once with phenol. The DNA fragments were precipitated with ethanol and resuspended in 50 μl of one quarter XE buffer. The sample was layered on to a 0.5 cm × 12 cm cylindrical gel of 0.7% agarose–2.2% acrylamide[36] and electrophoresed for 12 h at 50 V. The gel was stained and photographed as in Fig. 1. The gel was sliced into 1 mm sections and each fraction placed into 3 ml of Aquasol (New England Nuclear). The slices were soaked overnight and counted. Arrows refer to peak positions of the Ad2 DNA markers. The amount of DNA in each peak I–V is given in the text and is the average of five such measurements.

Restriction endonuclease cleavage

Bovine kidney cells were grown in tissue culture and the total DNA was labelled and extracted as is described in the legend to Fig. 1. The DNA thus extracted was of high molecular weight as the mean sedimentation coefficient as measured by centrifugation in a neutral sucrose gradient was 60S. Satellite I DNA has a density of 1.716 g ml^{-1} (refs 16, 17) in neutral CsCl and was isolated from the bulk of the DNA, which has a mean density in CsCl of 1.700, by both Ag^+–Cs_2SO_4 and CsCl equilibrium centrifugation techniques[16–19]. After the isolation of this satellite, the DNA was digested to completion with the restriction enzyme EcoRI (see ref. 20 for nomenclature of restriction enzymes), which cleaves DNA at the sequence

$$\begin{array}{l} \downarrow \\ {}^{5'}\mathrm{GAATTC}^{3'} \\ {}^{3'}\mathrm{CTTAAG}^{5'} \\ \uparrow \end{array}$$

(ref. 21). The products of this digestion were then analysed by electrophoresis through 1.4% agarose gels. Figure 1*a* is a photograph of a slab gel after electrophoresis and staining of the DNA with ethidium bromide and it illustrates that the material migrates in one discrete band. More than 95% of the radioactivity in satellite I DNA is recovered in this band. The molecular weight of this band of DNA was calculated to be 9.2 × 10^5 (± 6%), from its relative mobility to the EcoRI fragments of adenovirus 2 DNA[22] (Fig. 1). The molecular weight of EcoRI cleaved satellite I DNA indicates that within this DNA at points every 1,400 base pairs is the sequence $\frac{\mathrm{GAATTC}}{\mathrm{CTTAAG}}$. A partial digestion of the satellite DNA confirms the tandem nature of this repeat. If the enzyme digestion is stopped before completion, multiple bands of DNA are seen after electrophoresis whose molecular weights form an arithmetical progression (Fig. 1*b*). The monomer,

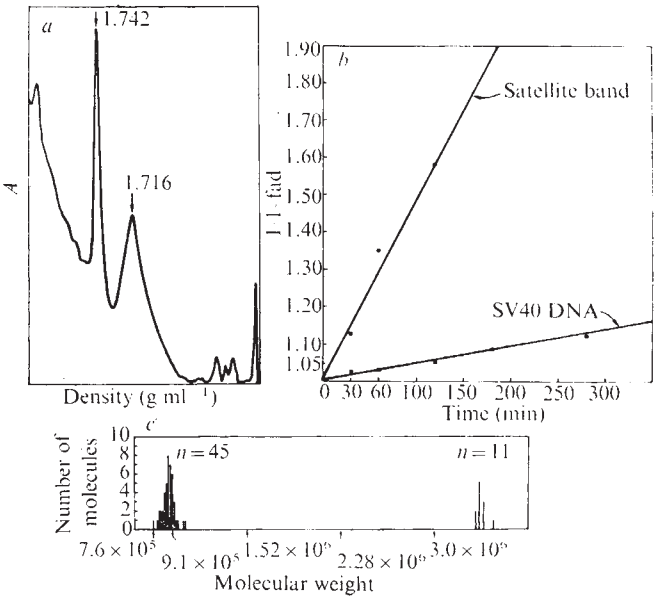

Fig. 3 Physical characterisation of satellite I DNA. *a*, Total bovine ^{32}P DNA was digested with EcoRI and electrophoresed as in Fig. 2. Fractions corresponding to peak I (fractions 66–69) were pooled and the DNA eluted by electrophoresis as described in refs 35 and 36. 0.5 μg of this DNA was centrifuged to equilibrium in CsCl at 44,000 r.p.m. in a Spinco Model E centrifuge. A densitometer trace of the *A* profile is shown. The density of 1.716 g ml^{-1} was calculated relative to SPOI DNA $\rho = 1.742$ g ml^{-1} using $1/\beta = 8.4 \times 10^{-10}$ c.g.s. units. *b* Renaturation kinetics of this ^{32}P satellite DNA were measured relative to ^{3}H-SV40 DNA by hydroxylapatite chromatography (Bio-Rad HTP) fad = fraction of DNA binding to HAP as double strands. 0.2 μg of the ^{32}P satellite DNA (specific activity 11,000 c.p.m. μg^{-1}) plus 0.42 μg of ^{3}H- SV40 DNA (40,000 c.p.m. μg^{-1}) were combined in 100 ml of 0.001 M Tris *p*H 7.9 + 0.025 M NaCl. 50 μl of 1 M NaOH were added and the solution boiled in a sealed capillary tube for 25 min to fragment the DNA. The solution was then neutralised with 50 μl of 1 M HCl and brought to 2.2 ml with 0.035 M phosphate buffer + 0.4% SDS. 0.5 ml were taken at the specified times and the fraction of molecules that were double-stranded was measured by chromatography at 60° C. Total eluants from the 0.14 M phosphate buffer and the 0.40 M phosphate buffer washes were precipitated separately with trichloroacetic acid and filtered on to Millipore HAWP filters. The ethanol washed filters were counted in Aquasol. The slopes of the lines differ by a factor of 11. Since the SV40 DNA is 2.1 times more concentrated than the satellite DNA, the kinetic rate constants differ by a factor of 23. *c*, Histogram of molecular weights of the EcoRI cleaved satellite I DNA: total high molecular weight satellite I DNA was digested with EcoRI as described in Fig. 1. The reaction mixture was extracted with phenol and the DNA precipitated with ethanol at −20° C. The precipitate was resuspended in 0.01 M Tris *p*H 8 buffer containing SV40 circular DNA. Molecular lengths were measured by electron microscopy as described in ref. 36. The lengths were converted to molecular weights by assigning a molecular weight of 3.4×10^{6} daltons for circular SV40 molecules.

dimer, trimer, tetramer and pentamer molecular weights of the 9.2×10^{5} dalton repeat were resolved.

The molecular weight of the EcoRI digested satellite DNA was measured by electron microscopy. A histogram of molecular weights is given in Fig. 3*c*. Assigning a molecular weight of 3.4×10^{6} for the circular SV40 DNA which was added as a marker, the EcoRI digested satellite had a molecular weight of 8.9×10^{5} (± 3%). There is then excellent agreement between the electron microscope and electrophoretic measurements of this repeat length.

Because satellite I DNA was cleaved to a uniform length by EcoRI, it seemed likely that cleavage with this restriction enzyme would provide a simple and quantitative procedure for the satellite isolation. The 1,400 base pair repeat in satellite I can indeed be detected in the EcoRI digestion products of the unfractionated total bovine DNA (Figs 1*a* and 2). When the total DNA is digested to completion with the endonuclease, a heterogeneous population of DNA molecules is obtained. Within this distribution one can clearly distinguish four bands of DNA, whose molecular weights are: 1.35×10^{6} (III); 10^{6} (II); 9.2×10^{5} (I); and 5.1×10^{5} (V) daltons (Fig. 1*a*). The source of the DNA did not influence these molecular weights as commercial calf thymus DNA (Worthington) yielded bands with DNA at identical molecular weights.

The amount of material in each of the bands was measured by digesting ^{32}P-labelled bovine DNA with EcoRI and analysing the fragments by electrophoresis. The distribution of ^{32}P-DNA, together with the positions of internal markers are shown in Fig. 2. The percentage of the total DNA under each band was calculated to be: 0.7% (IV), 0.5% (III), 1% (II), 6.7% (I), 0.1% (V) respectively for the peaks from highest to lowest molecular weight. The DNA from the peak labelled I was cut from a separate gel and eluted by electrophoresis. This DNA was identified as satellite I on the basis of the following criteria: (1) its buoyant density in a neutral CsCl gradient of 1.716 g ml^{-1} (see Fig. 3); (2) its relative amount in the genome of 6.7%; (3) its coelectrophoresis with EcoRI cleaved satellite I DNA; and (4) its renaturation kinetics being indistinguishable from that of satellite I (see below).

It is well known that the rate of reannealing of a unique sequence DNA is directly proportional to the complexity of the sequence[23,24]. Therefore, if the satellite I DNA were composed of a unique repeating unit 1,400 base pairs long, it should renature at a rate roughly four times faster than that of SV40 DNA, allowing for the fact that there is an 18% difference in G–C composition between the two DNAs. A comparison of the rates of renaturation of denatured SV40 DNA and denatured satellite I DNA is shown in Fig. 3*a*. The total kinetic complexity of the satellite as measured by renaturation kinetics is 200–250 base pairs. A complexity of 200 base pairs for the satellite I must mean that the 1,400 nucleotide repeat is internally repetitious. Based upon what is known about the structure of other satellites[11–14], a likely hypothesis is that the repeat unit has a short 8–20 base pair substructure with enough divergence in this basic sequence to cause an apparent complexity of 200–250 base pairs. The picture of this satellite then emerges as a repeating unit within a repeating unit. The EcoRI sequence repeat could be again part of a larger repeating unit containing the 1,400 base pair unit an integral number of times. In view of this, it was interesting to digest bovine satellite I DNA with another restriction enzyme.

At least two specific endonuclease activities can be isolated from *Haemophilus influenzae* serotype d. Hind II cleaves at the sequences

↓
5′ -GTPYPUAC-
-CAPUPYTA- 5′ (ref. 25). Hind III does not cleave satellite I
↑

(Fig. 4*b*). When high molecular weight satellite DNA is digested with Hind II or the combined enzymes, the DNA is degraded to two fragments of 7.4×10^{5} and 1.25×10^{5} daltons as measured by electrophoresis through 4% polyacrylamide gels (Fig. 4*a*). The same two bands can be seen in the Hind II + Hind III digest of total bovine DNA (Fig. 4*a*). The sum of the molecular weights of these two bands is 8.65×10^{5}. If the EcoRI repeat were part of a larger unit, two other enzymes might be expected to give fragments whose additive molecular weights indicated a greater complexity. There is a 3% difference between this estimate of the repeat length and the electron microscope length of the EcoRI repeat. A second enzyme system then indicates that there is a repeat unit every 1,400 base pairs within this satellite.

Restriction map of satellite

Within this repeat unit it is now possible to orient the EcoRI cleavage sequence and the two Hind II cleavage sites by comparing the products of digestion of the enzymes working

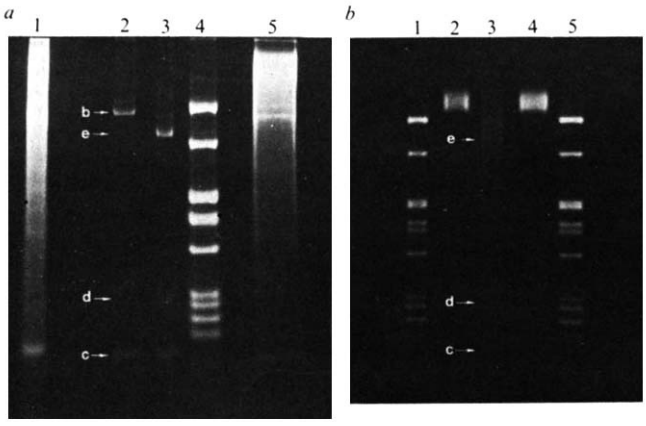

Fig. 4 Cleavage of satellite I DNA with Hind restriction enzymes. *a*, (1) total bovine DNA (1 μg) digested with Hind II + III restriction enzymes. (2) High molecular weight satellite I DNA (0.5 μg) digested with Hind II + III. (3) Satellite I DNA (0.5 μg) cleaved with EcoRI then with Hind II + III. (4) SV40 DNA (2 μg) cleaved with Hind II + III. (5) Total bovine DNA cleaved with Hind II + III. The Hind enzymes were isolated as described in ref. 25. The DNAs were cleaved in buffer containing enzyme and 0.006 M $MgCl_2$ + 0.01 M Tris *p*H 7.4 + 0.1 mM DTT for 12 h at 37° C. After digestion and extraction with phenol, the DNA was precipitated with ethanol and resuspended in 20 μl of 1/4 × E buffer containing 20% sucrose. The samples were then placed on to the 4% polyacrylamide slab gel. The gel contained 4% acrylamide (Bio-Rad), and 0.2% N'N'-methylene-bis-acrylamide (Eastman Kodak) and was polymerised by the addition of NNN'N'-tetraethylenediamine and ammonium persulphate. Electrophoresis was for 6 h at 50 V. Molecular weights were measured relative to the SV40 fragments[37]. b in position 2 – 7.4 × 10^5 daltons, c in positions 1,2,3,5 = 1.2 × 10^5 daltons, e in position 3 = 5.7 × 10^5 daltons, d in position 3 1.8 × 10^5 daltons. Other bands in 2 and 3 are partial cleavage products. *b*, EcoRI cleaved satellite I DNA was digested with Hind III (a gift of Dr R. Roberts). The Hind III + EcoRI cleaved DNA in position 2 has the same mobility as RI cleaved satellite in position 4. An aliquot of the Hind III reaction mixture was taken and to it added the Hind II enzyme. The DNA from this experiment is shown in position 3. The SV40 DNA fragments obtained by cleavage with Hind II + III are shown in positions 1 and 5.

separately and together on the DNA. Satellite I DNA was cut with EcoRI. After extraction with phenol, the DNA was redigested with the Hind II enzyme. The products of this double enzyme digestion were DNA fragments of three molecular weight classes as measured by electrophoresis in 4% polyacrylamide gels (Fig. 4*a*, *b*). The molecular weights are: 5.7×10^5, 1.8×10^5 and 1.2×10^5 daltons. That there is a true repeating unit within the satellite is further indicated by the finding that the amount of DNA in each one of these three bands is proportional to its fraction of the repeat length. Thus, the relative amounts of radioactivity in the three bands are 65%, 20% and 15%.

An unambiguous map of these restriction sites (see Fig. 5) within the repeat unit can be deduced from the following: (1) The distance between the two Hind II restriction sites is 1.2×10^5 daltons. This accounts for the 1.2×10^5 dalton fragment (c) seen in both the double enzyme (EcoRI+Hind II), and the Hind II digestions. (2) The RI cleavage site must be 1.8×10^5 daltons from one of the Hind II sites. This accounts for the 1.8×10^5 daltons band (d) resulting from the double digestion (EcoRI+Hind II). (3) The 7.4×10^5 dalton band (b) arising from the Hind II digestion is cut once by EcoRI to yield 5.7×10^5 (e) and 1.8×10^5 (d) dalton bands. These two bands orient the RI site with respect to the Hind II sites.

A conclusion of the restriction map shown in Fig. 5 is that the repeat unit detected is tandemly repeated (. . . ecdecdecd . . . .). Inverse tandem repeats (. . ecddce . . .) can be eliminated as this would be detected as a band at 3.6×10^5 daltons (d + d) in the Hind II cleavage experiments.

Evolution of satellite I DNA

The evolution of satellite DNA poses a paradox. On the one hand, apparent fluctuations of satellite content in related species are quite well documented[5,12,26]. In the genus *Dipodomys* the amount of density satellite DNA ranges from barely detectable in *Dipodomys spectabilis*, to more than half the nuclear DNA content in *Dipodomys ordii*[27]. In general, it is not clear as to whether these sequences are lost from the basic genome or through translocations and mutations become unrecognisable. By contrast to this interspecies variability, is the remarkable homogeneity of repeats that can exist within one satellite[14]. Furthermore, it is clear that different satellites within the same organism[14,28] and in different species[14,29] are related to each other. The problem then in satellite evolution is to account for apparently rapid changes in content and in sequence, while allowing for homogeneity within tandem arrays and sequence relationships between different satellites.

Two basic mechanisms for satellite evolution can be considered, one being unequal crossing over and the other DNA replication. From mathematical considerations, Smith[30] has shown that if tandem arrays of sequences become heterogeneous, unequal crossing over will tend to homogenise the array. By this process of unequal crossing over, the sequence of one repeat unit will eventually dominate the cluster. With regard to the origin of arrays, unequal crossing over can also clearly increase or decrease the size of an array by a single recombination.

Mechanisms of satellite DNA evolution based on DNA replication must address themselves to the frequency of replication origins in this DNA. In the case of the bovine satellite I DNA which comprises 7% of the genome, there must be 144,000 copies of the 1,400 base pair repeat. It seems reasonable to assume that the 2,000–3,000 tandem repeats of the 1,400 base pair unit that are present on each autosomal chromosome contain interspersed within them multiple sites for the initiation of DNA synthesis. Alternately, each 1,400 nucleotide repeat may contain such a signal. Reciprocal intrastrand recombination in direct tandem repeats will favour excision and the formation of independent rings. These recombinations, in fact, seem to occur because polymorphism in human centric-heterochromatin indicating deletions in satellite DNA content have been shown to be widespread[31,32]. If a satellite DNA circle formed by such an excision contains a sequence for the initiation of DNA synthesis it may undergo rolling circle replication and amplification. That the enzymes needed for such a process are available in higher cells at meiosis has been shown, as Hourcade *et al.*[33] and Bird *et al.*[34] have demonstrated that ribosomal DNA

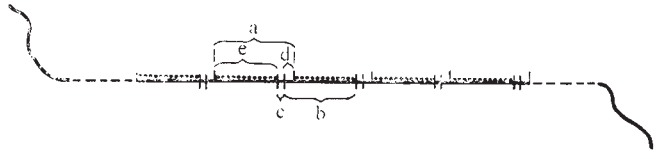

Fig. 5 Restriction map of satellite I DNA. Assuming that this satellite is approximately equally distributed on all 58 autosomes[16] there are 2,000–3,000 repeats of unit (*a*) in each chromosome. This represents the spacing of the EcoRI restriction sequence. Because more than 95% of the satellite is cleaved to this molecular weight (a) there can be no interspersion of other sequences not in this repeat unit at the level of once every 20 repeat lengths. The two Hind II restriction sequences are spaced by a distance c from each other and a distance d from the EcoRI sequences. When the satellite I DNA is cut with EcoRI and then the Hind II restriction enzyme fragments e, c and d are generated. When the satellite DNA is cut with the Hind II enzyme alone, fragments b and c are generated. Partial cleavage products of the double enzyme experiment are: e + c, and c + d. Partial cleavage of Hind II on high molecular weight satellite yields a fragment of size b + c. No polarity to the strand shown can be deduced from the data presented. Therefore, the repeat unit can either be ($^{5'}$–ecdecd–$^{3'}$) or ($^{3'}$–ecdecd–$^{5'}$).

is amplified by a rolling circle mechanism in *Xenopus* oocytes. The product of this rolling circle replication is a tandem array of identical repeats. To become part of the permanent germ line of the organism, the amplified DNA must subsequently recombine back into a chromosome. This model could account for the origin of new satellite sequences since the repeat that is excised might be sufficiently diverged from the bulk of the satellite DNA. The existence of a previously undetected long range order in satellite DNA is a strong prediction of this model.

The data presented here indicate that two stages were involved in the origin of satellite I DNA in the bovine genome. The first stage involves the small duplications indicated by the renaturation rate to develop the 1,400 base pair repeat, which may have arisen either by a DNA replication process or by unequal crossing over. The second stage involves the production of tandem arrays of this repeat. Should unequal crossing over occur at any point of small homology within the tandem array, regularity of the higher order repeat will be destroyed, because the 1,400 nucleotide units would presumably pair in a staggered orientation. Perhaps the mechanism of recombination could place a restriction upon such staggering (for example, the joining between recombining strands might always include one repeat unit in phase). In view of the contrived nature of this restriction, DNA replication schemes, specifically the rolling circle, seem more feasible for this second process.

Whatever the mechanism involved, amplification may occur rarely. After the satellite DNA has been incorporated into the genome, base changes, insertions and deletions will tend to obscure the homogeneity of the array. In a previous study[35], we have shown that the distribution of EcoRI sites in the mouse satellite is very irregular. We interpreted this to be an indication of this divergence process and that the correction of the repeats in this satellite is either an imperfect or a rare event. But, other restriction enzymes or more complete sequence data on the mouse satellite may indicate a long order organisation to this DNA. It will be important to know whether all satellite DNAs possess a regularity at the level of organisation reported here, or if this is particular to the bovine satellite I.

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letters to nature

Near absence of hard X-ray pulsations in Her X-1

We have previously presented[1] spectral results for Her X-1 in the energy range 20 to 100 keV and have set an upper limit of about 40% for the pulsating component. We have recently made further observations on this source with a telescope of much larger area and have obtained a longer exposure during its bright phase in the binary cycle (phase = 0.25) and almost in the middle of its 'on' period in the 35-d cycle (phase = 0.45 to 0.55). The new observations set an upper limit of 10% for the pulsating component in the energy range 20 to 45 keV which has important consequences on the models proposed for the X-ray emission features of Her X-1.

The balloon flight was carried out from Hyderabad, (17.3°N) on December 12, 1973. The balloon floated at an altitude of 4 mbar and Her X-1 was observed during 0536 to 0654 UT for 4,240 s.

The X-ray telescope, with a field of view 8° × 8°, comprised four NaI crystals each of area 97 cm² and thickness 3 mm,

mounted on an oriented platform. To obtain a long exposure on Her X-1 and also the background counting rate, the telescope was inclined at 17° to the zenith and programmed to point due north for 7 min and 8° east of north for the following 7 min; this cycle of orientation was continued throughout the flight. In-flight calibration of the detectors was carried out with a ^{241}Am source. Pulse heights in the energy range 21 to 125 keV were sorted into 15 energy channels and telemetered, together with aspect information. The time of occurrence of each event was recorded with an accuracy of 1 ms.

The procedure for evaluating the efficiencies as a function of time, and the reduction of spectral data, have already been described (ref. 1 and references therein). In the energy range 21.5 to 81.7 keV a total of 11,561 ± 1,122 excess counts attributable to Her X-1 were recorded in a total exposure of 5.7×10^5 cm^2 s. The spectral data and our earlier results are plotted in Fig. 1. The fluxes observed in the March experiment

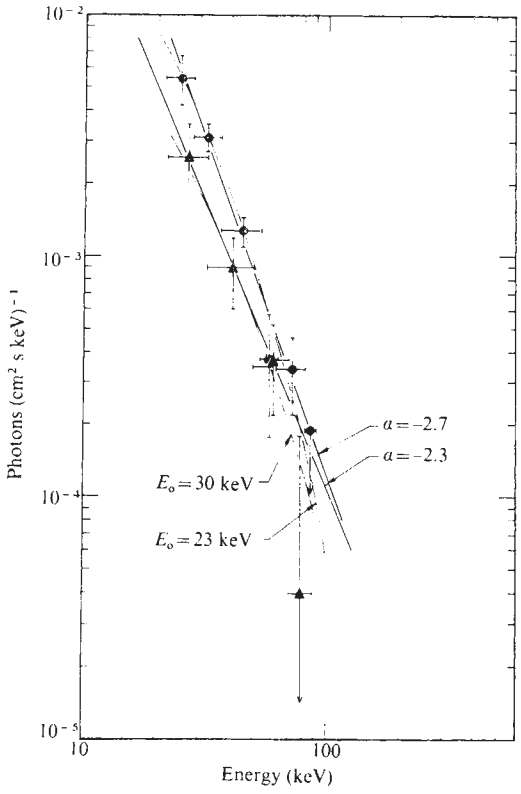

Fig. 1 Energy spectrum of Her X-1 in the hard X-ray band 20 to 90 keV measured in the experiments of March 9, 1973 and December 12, 1973. Best fits for power law (E^{α}) and plasma temperature (kT) for each of the two sets are also shown. ▲, March 9 (ref. 1); ●, December 12 (present work).

are about a factor of two lower than the fluxes observed in the December experiment. The December data can be fitted with either a power law with an exponent −2.7 or with a thermal spectrum characterised by a kT of 23 keV. The March results could be fitted with a power law of exponent −2.3 or a thermal spectrum with a kT of 30 keV. Combining the satellite observations below 20 keV and other balloon observations (Fig. 2) we find that a plasma temperature of 23 keV ($T = 2.7 \times 10^8$ K) fits reasonably well the entire data over an energy range 2 to 80 keV. The scatter in Fig. 2 arises because the observations were made at different phases of the binary and 35-d cycles. One significant feature is the steep fall in the intensity beyond 80 keV. Such a steepening was also reported by Ulmer *et al*[2,3]. The anomalously high value of Sharma *et al*[4] for intensities in the energy band 80 to 100 keV is not supported by our observations. If the intensity was really high it should have been seen with high statistical significance in our experiment. There is some evidence in the present experiment for variations

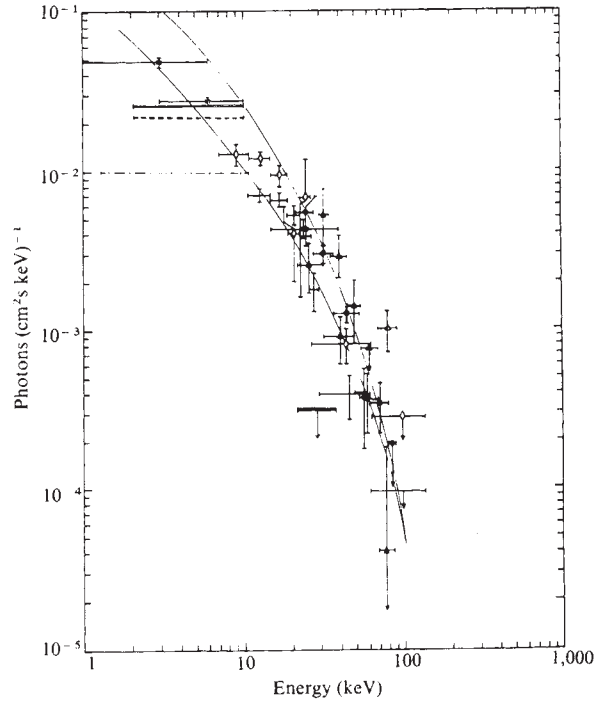

Fig. 2 Data on the energy spectrum of Her X-1 in the 1 to 100 keV interval, together with the fit for plasma temperature kT 23 to 30 keV as derived from our observations. Diamond and +, Refs 2, 3; —, Uhuru [5,6]; ▲, ref. 1; △, ref. 4; ×, ref. 9; ●, present work. Pulsation: – –, Uhuru; – · – · – ·, ref. 7; —, our work.

in intensity in the 20 to 60 keV band by a factor of about two within the time scale of observation of ~70 m.

In our experiment the source 3U1706+32 did have a considerable exposure when observations on Her X-1 were made. But the intensity of the source in the 2 to 6 keV band is 1/25 that of Her X-1 (ref. 5) and even if the spectrum is flat, the contribution in the 20 to 45 keV will be small compared with that of Her X-1.

Since most of the contribution from Her X-1 was recorded in the energy channel 21.5 to 45.2 keV, the timing data in this interval were analysed to find the fraction of the pulsating component. The data were cast into 20 phase bins and none of the phase histograms with periodicity ranging from 1,235 to 1241 ms revealed a peak larger than 2.5σ. The χ^2 was as low as nine for 19 degrees of freedom (95% confidence). The data were also analysed in shorter time stretches of ~10 min each, and no significant peaks were seen in this analysis. These observations lead to an upper limit of 10% for the pulsating component if the width of the pulse is less than 400 ms. The limit is about the same even if the pulse width is as short as 60 ms. The data was also subjected to a Fourier analysis using an FFT algorithm; the power density distribution (Fig. 3) does not reveal any appreciable power in any of the frequencies investigated. In view of the negative results the algorithm was further tested by analysing self-generated data modulated with a known frequency.

The present upper limit of 10% for the pulsating component in the 20 to 45 keV band is to be compared with the lower limit of 80% in the 2 to 6 keV band[6] and 60% in the 2 to 11 keV band[7]. Ulmer *et al.*[2] have remarked that their preliminary analysis of the OSO-7 data failed to reveal any pulsations in the 7 to 60 keV band but further analysis may allow them to detect pulsations. Kurfess and Crosa[8] found no pulsed emission at energies greater than 100 keV, and could set a 2σ upper limit of 3.4×10^{-3} photons cm^{-2} s^{-1} in the 0.1 to 10 MeV range assuming a duty cycle of 35%.

So there is no evidence for a pulsating component at energies greater than 20 keV. As shown earlier the spectrum of the

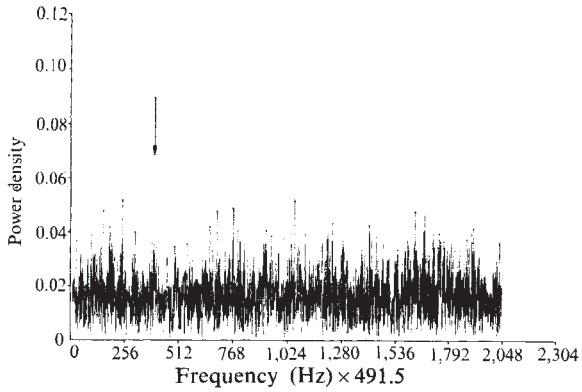

Fig. 3 Power density spectrum up to 2,048 Hz as obtained in the Fourier analysis of ~492 s of observation in the range 21.5 to 45.2 keV. The frequency corresponding to 1.24 s is shown by an arrow.

total X-ray emission over the entire energy range 2 to 80 keV can be fitted with a single plasma temperature of $kT = 23$ to 30 keV. The behaviour of the pulsating component as a function of energy is also shown in Fig. 2.

The possibility that the hard X rays arise from a different object close to Her X-1 can be ruled out from evidence[3,9] of the binary modulation and the 35-d cycle variation for the hard X-ray emission in phase with the modulation at lower energies.

The models which have been proposed to explain the features in the 2 to 10 keV range, regard Her X-1 as a rotating magnetised neutron star accreting matter from an eclipsing companion of larger size[10,14]. The influx of matter is constrained to a narrow region around the magnetic poles. The X-ray emission results from the transformation of the kinetic energy of the infalling matter into radiation which emerges preferentially in directions almost normal to the direction of the inflow. The observation of pulsed emission with the frequency of rotation is then a consequence of the direction of the magnetic axis being different from the rotation axis. The detailed pulse profile and in particular the pulse width, is determined by the angle between the two axes, the emission pattern and the direction of viewing. In such a model, if the hard X rays (20 to 80 keV) also originate from the same region as X rays of lower energy (1 to 10 keV) the pulsating fraction can be different only if the fan beam in hard X rays is considerably broader so as to reduce the extent of modulation during rotation. This, however, is contrary to the prediction of Davidson's accretion model[14] according to which the harder X rays come from a relatively narrow band at the base of the accretion funnel resulting in a narrower pulse width than in the X rays of 2 to 10 keV. Thus the present observations point to the need of a second component of X rays apart from the pulsating part.

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Direct observation of rock macerates

IN scanning electron microscopical (SEM) investigations of Precambrian sedimentary rocks, the risk of contamination during the preparation of rock macerates is extremely high. The technique described here and the use of stringent controls, have reduced to a minimum the degree of contamination by dust and microorganisms and, during preparation, enabled the recognition of artefacts from laboratory reagents.

In a preliminary study we used a dark, laminated, shaly sandstone from the Diabaig Formation of the Torridon Group, in north-western Scotland[1] (NG799605)[2]. This formation is believed to be older than $995 \pm 24 \times 10^6$ yr (ref. 3), and was selected because samples from other outcrops of this formation have already yielded microfossils[4-6].

Samples of rock were powdered with a hammer on a clean steel block and the powder was passed through a 600-μm sieve. Working in a sterile air flow, small samples of the powder were put into purpose-built wax stub tubes[7] in stoppered plastic test tubes, and treated with Schulzes' solution for 3 h to oxidise any exposed organic matter. Tests on spores of *Penicillium chrysogenum* showed that they were destroyed after only 30 min treatment; 3 h was, therefore, considered long enough to clean the

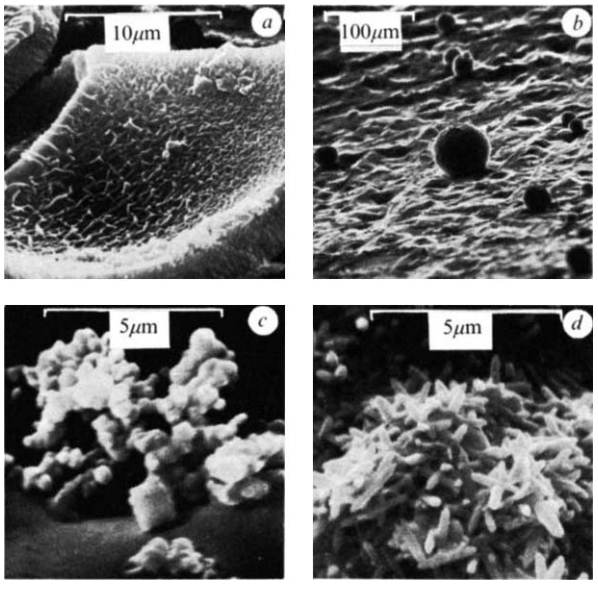

Fig. 1 *a*, Ornamented crust from the evaporation of laboratory distilled water; *b*, resin spheres from de-ionised water; *c*, residue after the reaction of hydrofluoric acid with silica; *d*, precipitated metal fluorides from a prepared rock macerate.

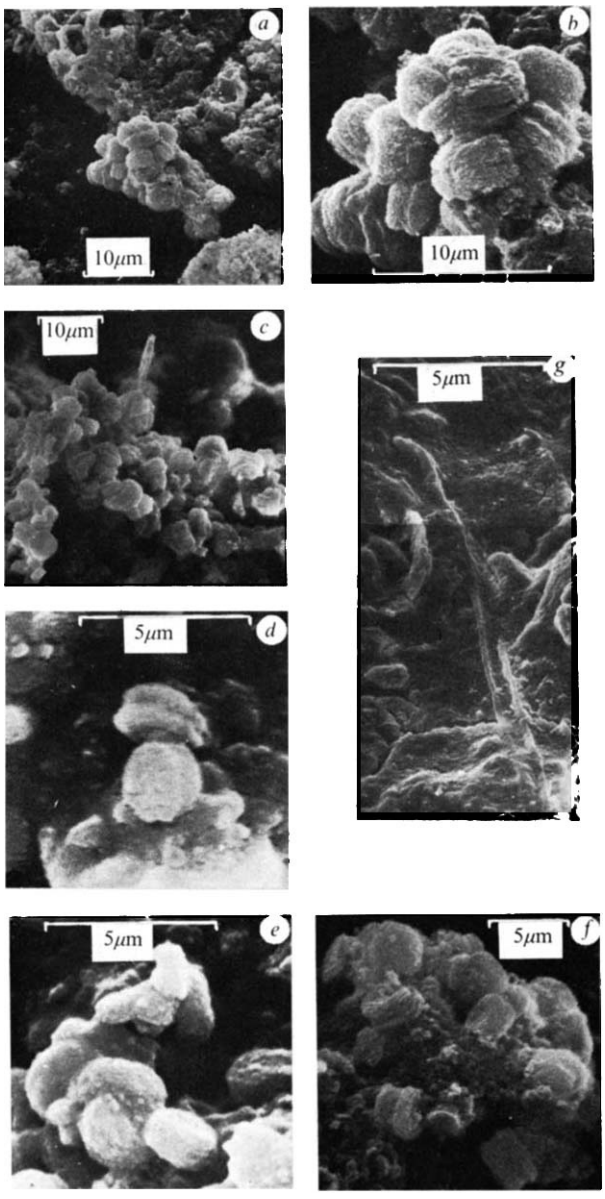

Fig. 2 *a–f*, Rounded microfossils (*b* is a close-up of *a*); *g*, filaments (composite micrograph).

sample. The samples were short centrifuged and washed until free of acid, and were then treated with 38–40% hydrofluoric acid for 6 h, centrifuging down the residues and changing the acid every 2 h. The final residues were short centrifuged and washed until free of acid, and the stub tubes were left to dry overnight in a clean vacuum desiccator. The following day, the residues were prepared[7] for SEM observations. Control preparations of all reagents were examined by SEM, revealing many artefacts (Fig. 1*a–c*). Laboratory distilled water was evaporated to form an ornamented crust (Fig. 1*a*), and de-ionised water carried over microspheres of resin (Fig. 1*b*). So to wash the residues, glass distilled water was de-ionised and then distilled again in a still cleaned with chromic acid. To determine whether pure silica could produce organised microstructures after reaction with hydrofluoric acid, a silica glass test tube was sacrificed, and when treated in the same way as the rock, structures were produced (Fig. 1*c*).

Examination of rock residues in the SEM revealed considerable amounts of organic and mineral debris, together with organised microstructures. Figure 1*d* probably represents a precipitate of fluorides[8], and we believe the structures of Fig. 2*a–g* to be the remains of microorganisms, a judgement based on their superficial resemblance to extant genera of algae[9] and other described fossil forms[10]. We consider it unlikely that these structures were produced by inorganic precipitation or by recrystallisation.

We believe that these preparations are of technical interest in that they demonstrate the feasibility of using the SEM wax stub tube for micropalaeontological maceration when it is necessary to reduce contamination to a minimum. We have also found the stub tube invaluable in monitoring the artefact content of distilled water, and of active reagents such as hydrofluoric acid. Although we do not suggest that the stub tube should replace other mounting techniques in scanning electron microscopy, we submit that it can be a useful tool in the critical study of organic microfossils.

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3,500-Myr-old granite in southern Africa

A GEOCHRONOLOGICAL study of the margin between the Rhodesian Craton and the Limpopo Belt in southern Africa has yielded the first conclusive evidence for the preservation of very old crustal material on the Rhodesian Craton. An age of 3,520±260 Myr has been obtained from a seven-point Rb–Sr whole rock isochron for samples of the Mushandike Granite, which intrudes tonalite gneisses near Fort Victoria (30° 50′E, 20° 04′S) in south-eastern Rhodesia. Its relation to the Bulawayan Group of the Victoria Schist Belt is uncertain. The possible presence of an intrusive contact of the granite within these rocks points out the need for a re-examination of the evidence for the widely accepted maximum age for the Bulawayan of 3,300–3,400 Myr (ref. 1).

The Mushandike Granite and its contacts with surrounding rocks have been described by Wilson[2,3]. For the most part, the 'granite' is a medium grained, massive granodiorite. It has undergone shearing and faulting since emplacement; the effects of that are especially intense in the southern and western parts, whereas in the northern and central parts little or no deformation has occurred. Evidence that the Mushandike Granite intrudes the Tonalite Gneiss Complex is strong. Wilson[2,3] reported xenoliths, typical of the

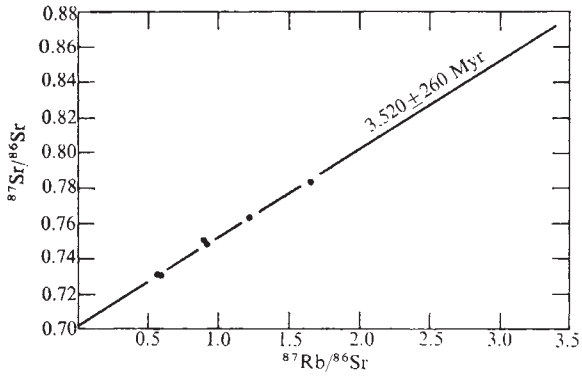

Fig. 1 Rb–Sr whole rock isochron for the Mushandike Granite.

surrounding gneisses, both in the centre of the granite and along its northern margin. In the north and south-east, the granite forms a lit-par-lit contact zone with the Gneiss Complex. Banding in the gneisses is roughly parallel to the contact along both margins, but tongues of granite cut across the banding in the north[3].

Along the southern margin of the granite, phyllites and amphibolites, assigned to the Bulawayan Group of the Victoria Schist Belt[2,3], occur. Shearing and faulting have obscured the original nature of the contact. Wilson[2,3] considers the granite to be post-Bulawayan. A narrow tongue of deformed granite, which can be traced into recognisable Mushandike Granite, occurs in the schist belt, suggesting an intrusive relationship. In one location the contract cuts across the trend of the phyllites. The occurrence of randomly oriented andalusite in the phyllites, and of cordierite and anthophyllite in the amphibolite, could be a result of contact metamorphism by the granite. Without a better understanding of the effects of the intense deformation in the contact zone, this evidence cannot be taken as conclusive; the present contacts could all be tectonic.

The Rb–Sr whole rock isochron ($\lambda\ ^{87}Rb = 1.39 \times 10^{-11}\ yr^{-1}$) for the Mushandike Granite, gives an age of 3,520±260 Myr (Fig. 1); the analytical data are shown in Table 1. The

Table 1 Analytical data

No.	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}Rb/^{86}Sr$	$^{87}Sr/^{76}Sr$
1	71.8	354	0.587	0.7307
2	69.2	340	0.590	0.7305
3	65.5	209	0.907	0.7504
4	86.3	268	0.934	0.7487
5	63.9	151	1.22	0.7635
6	111	194	1.65	0.7840
7	107	93.9	3.32	0.8611

seven analysed samples are all from the centre of the granite, away from the effects of deformation. There is evidence of some geological error in the data, and the quoted error in the age is that given by the isochron calculations McIntyre II and York I (ref. 4). Errors are quoted at the 95% confidence level.

The calculated age is important in establishing conclusively the preservation of ancient crustal material on the Rhodesian Craton. It supports the findings of Robertson[5] who, on the basis of a detailed study of Rhodesian leads, postulated a ~3,500 Myr event on the Rhodesian Craton. He suggested that the event was associated with the formation of granitic crustal segments as well as with pre-Bulawayan lavas.

On the basis of field relationships, the Tonalite Gneiss Complex must be older than the Mushandike Granite. Preliminary isotope data for tonalite gneisses from the town of Fort Victoria, 10–15 km to the east suggest, however, that they may not record a significantly older isotopic age. The points do not define an isochron, showing considerable scatter, but they show no systematic deviation relative to the Mushandike Granite isochron. That may mean that correlations in the Gneiss Complex cannot be made over the distance involved, or that the intrusion of the Mushandike Granite was part of a widespread event which overprinted any isotopic record of the early history of the gneisses in the area. It is also possible that the emplacement of large bodies of granite north and south of the Victoria Schist Belt about 2,650 Myr ago[1] may have caused some isotopic mixing in the Fort Victoria gneisses at that time.

The ambiguity of the contact between the Bulawayan Group of the Victoria Schist Belt and the Mushandike Granite points out the need for reliable and precise age determinations on material from the schist belts themselves and on granitic bodies which have reasonably clear relationships to the schist belts. The ambiguous nature of the Mushandike–Bulawayan contact and uncertainty in the available, relevant age data mean that the age of the Bulawayan is now in question. The work of Robertson[5] suggests that most of the volcanics assigned to the Bulawayan Group are younger than 3,500 Myr, because leads recording a ~3,500 Myr event are generally restricted to pre-Bulawayan rocks. Three K–Ar determinations on muscovite from the pre-Bulawayan Que-Que Granite, about 160 km north north-west of Fort Victoria (29° 48′E, 18° 53′S) have given an age of 3,300–3,400 Myr (ref. 1). Although this clearly needs to be confirmed by other dating methods, it has, in the absence of other data, been widely accepted as an approximate maximum age for the Bulawayan.

Elsewhere in southern Africa, rocks older than 3,000 Myr have been found in the Barberton Mountain Land of the Kaapvaal Craton[6]. Of particular interest here is the new evidence, from Rb–Sr analysis of mineral fractions, that basalts of the Lower Onverwacht Group have an age of 3,500±200 Myr. These rocks have been correlated with pre-Bulawayan volcanics on the Rhodesian Craton[7]. The Ancient Tonalite Gneisses which intrude the Lower Onverwacht have a Rb–Sr whole rock age of 3,440±300 Myr (ref. 8).

No definite conclusions about the Rhodesian Craton can be drawn on the basis of Kaapvaal Craton age data. Chronological equivalence cannot be inferred from lithostratographic correlations between cratons, or even between different regions of a single craton. Until further work is done on Rhodesian material, the chronology of the Bulawayan Group remains uncertain.

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Thermal model for origin of granitic batholiths

THE origin of granitic batholiths has been reviewed in the light of our understanding of the processes operative at destructive plate margins[1]. The thermal patterns at island arcs suggest that significant amounts of thermal energy must be released within downgoing oceanic plates, and that this energy may be transferred to the surface behind island arcs. In these regions heat flow values are abnormally high and andesitic volcanism is present. The high heat flow may be produced because the normal geothermal gradient is enhanced by penetrative convection of magmatic material which is derived from the downgoing plate. Many large granitic batholiths, such as those in Chile, are localised at plate margins, and some authors have proposed that the Sierra Nevada batholith originated in a similar setting along the continental margin in the downwarped portion of the lower crust. Dickenson[2] suggested that the magma for the batholiths was produced by partial melting within the downgoing plate, and was subsequently emplaced into the overlying continental crust. Bateman and Dodge[3], and Brown[1] suggested that emplacement into the lower crust, of andesitic material derived from the mantle, causes partial melting of crustal material, with the mixed product forming the magma for the granitic batholiths.

Experimental studies of the generation of granitic melts[4,5] from metamorphic rocks have shown that melts of granitic composition will occur under conditions of crustal pressure at temperatures of about 700–750° C provided that water is released by dehydration reactions. A granitic melt may be produced by partial melting of the crust at depths of 25–30 km, whereas granodiorites and diorites may be derived from partial melting at higher temperatures (800–950° C) and deeper crustal levels (30–45 km)[1]. At the continental margins where the crust is 40–50 km thick, the radioactive content and the thermal conductivity of crustal rocks, together with the heat flow values, suggest that the lower crust is at a temperature of around 700° C, although higher temperatures may be achieved locally, adjacent to mantle-derived magma. Using thermal considerations I have investigated the extent of partial melting in sialic crust adjacent to a deep seated intrusion.

The thermal effects which a magma induces in country rocks are considered first. The cooling of a magmatic body that is assumed to be instantaneously emplaced may be expressed by the time-dependent equation for heat transport in one dimension:

$$K(\delta^2 T/\delta Z^2) = \rho c_p (\delta T/\delta t) \qquad (1)$$

where K is the thermal conductivity, ρ is the density, and c_p is the specific heat. Solutions to the equation extended to handle partial melting, can be approximated by finite difference techniques: the governing difference equation solved for temperature is

$$T(Z, t + \Delta t) = [1 - 2K\Delta t/\rho c_p (\Delta Z)^2]\, T(Z, t) + K\Delta t/\rho c_p (\Delta Z)^2$$
$$[T(Z - \Delta Z, t) + T(Z + \Delta Z, t)] \qquad (2)$$

where $T(Z,t + \Delta t)$ is the temperature at time $t + \Delta t$ at a location Z; Δt is the time increment; and ΔZ is the distance increment. The finite difference approximation of the heat conduction equation for two different media, country rock and magma, requires that the respective values of ρ, K and c_p for each medium are used in equation (2). A boundary layer equation is then adapted for the contact between country rock and magma.

I have modified the numerical techniques to handle partial melting. Partial melting is typically an endothermic process, and the total amount of heat of fusion, H, necessary to melt a material is a function of the rock composition. The total melting of basalt may absorb 400 calorie g^{-1}, and 10% partial melting may absorb, proportionately, 40 calorie g^{-1}. The latent heat of fusion of rocks of granitic composition is about 50 calorie g^{-1} (ref. 6). Thus, if it is assumed that a metamorphic rock is composed of 50% granitic material with the other 50% of mafic composition, to melt the granitic fraction of this rock would require a minimum of 25 calorie g^{-1}. It can be assumed that H is absorbed linearly over a range of melting, or as assumed here, the melting process can be simulated by absorption of H at a constant temperature, similar to the melting of a single component chemical species. Although the melting of a metamorphic rock is probably more complicated, that assumption is probably a reasonable description of the fusion process[7].

If a point, Z, reaches the temperature of melting, TM, it absorbs heat according to the equation

$$\Delta H = c_p \Delta T$$
$$\text{where } \Delta T = T(Z, t + \Delta t) - TM. \qquad (3)$$

The temperature of the point, Z, is TM until the total heat of fusion, H, has been absorbed. Once H is absorbed, the point responds according to equation (2). The absorbed heat will be released—equation (3)—as the temperature falls to TM and solidification begins; the heat of solidification is assumed to be equal to H. Conservation of energy is maintained throughout the process.

A simple geological model may best illustrate the effect of partial melting in country rocks adjacent to a magmatic body in the lower crust. The following parameters are used: $TM = 700°$ C; ρm (density of magma) $= 2.7$ g cm^{-3}; ρc (den-

Table 1 Partial melt zones around emplaced magma

Width of magma (km)	Initial temperature of country rock (° C)	TM of country rock (° C)	H (calorie g^{-1})	Total width (both sides) of zone of partial melting (km)
2	500	700	50	0.8
4	500	700	50	1.6
6	300	700	50	1.2
6 (no convection)	400	700	50	0.9
6	400	700	50	1.8
6	500	700	50	3.0
6	500	700	100	2.4
6	500	800	50	1.2
6	500	800	100	1.0
6	600	700	50	5.2
6	600	800	50	2.2
10	500	700	50	4.0

sity of country rock) = 3.3 g cm^{-3}; Km = 0.01 calorie cm^{-1} s^{-1}; Kc = 0.007 calorie cm^{-1} s^{-1}; T_m = 1,200° C (temperature of magma); H = 50 calorie g^{-1}; m = magma; c = country rock. Consider a slab shaped magma, 4 km thick, emplaced into a lower crust with an original uniform temperature of 500° C. It is likely that a mafic magma of this size, and a viscosity of 10^6 poise, will convect naturally[8]. One of the effects of convection within the magma will be to raise the temperatures in the country rocks above the maximum which would have been achieved if the magma was cooling without convection (Table 1). It is assumed that the temperature distribution within a freely convecting magma will be governed by the adiabatic law so that the temperature may vary with depth by anything from 0.3 to 1° C km^{-1}. Therefore the change in temperature from the top to the bottom of a convecting body 4 km thick will be only 2° to 4° C. The temperature distribution within and outside the magma at different times, for one half of a symmetrical model, is shown in Fig. 1. The temperatures reflect the heat

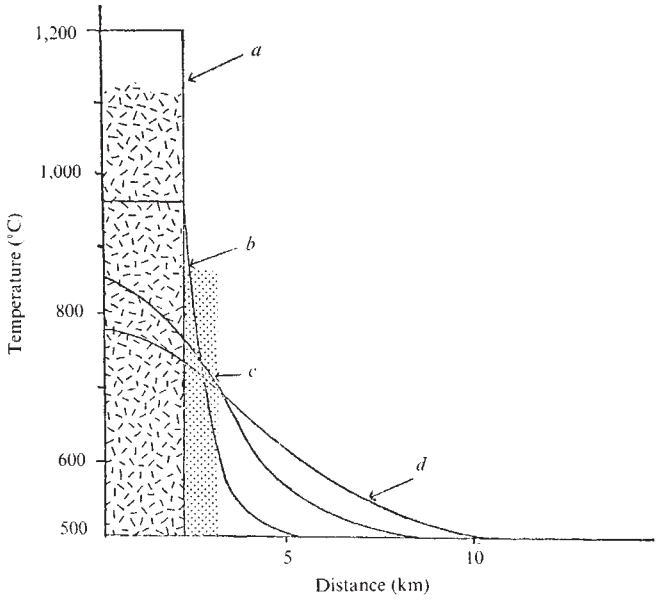

Fig. 1 The temperatures around a symmetrical magma 4 km thick: *a*, at emplacement; *b*, after 2 × 10^4 yr; *c*, after 10^5 yr; *d*, after 2 × 10^5 yr. Stippled area is the zone of partial melting. TM = 700° C; H = 50 calorie g^{-1}; initial temperature of the magma = 1,200° C. At T_m = 900° C convection ceases and the magma cools by conduction.

(H = 50 calorie g^{-1}) which is absorbed in the country rock as a result of melting once the melting temperature, TM (700° C) has been reached. After 40,000 yr, 0.8 km of country rock would be partially melted and the total partial melt zone on both sides of the magma chamber would be 1.6 km wide.

As most magmas derived from the mantle will have properties that do not vary greatly from those assumed here, the size of the partially melted zone around an intrusive will be principally a function of the thickness of the intrusive and of the initial temperature and composition of the country rock. Table 1 shows the width of zones of partial melting for several different examples. In each of these examples the parameters had the values already given here, and except in the cases specified in Table 1, convection within the magma was assumed.

Using H = 50 calorie g^{-1}, a magma 6 km thick intruded into country rock which is at 600° C, would partially melt a zone 2.6 km thick on each side. Within the zone of partial melting the amount of liquid granitic melt is a direct function of the original composition of the metamorphic rock. Thus, for example, with a metamorphic rock which was originally 50% granitic and 50% mafic, the zone of partial melt may be 50% liquid. A slab of magma 6 km thick, emplaced into country rock which is at 400° C, would have a zone of partial melting with a total combined width on both sides, of 1.8 km. If a magma were emplaced into a metamorphic rock of mafic composition with little granitic fraction, the width of the zone of partial melting would be reduced. This can be illustrated by taking H equal to 100 calorie g^{-1} instead of 50 calorie g^{-1}. The width of the zone of partial melting is reduced from 3.0 to 2.4 km thick (Table 1), but the amount of partial melting is still substantial. For a case in which the country rock has a higher temperature of melting (TM = 800° C) and a larger heat of fusion (H = 100 calorie g^{-1}) than the magma, the total width of the zone of partial melting on both sides of the magma would be only 1.0 km. As these properties may be more similar to a mafic metamorphic rock, the partial melting of metamorphic rock around a magma is probably limited. These results (Table 1) indicate that large portions of country rock with some fraction of granitic composition, can be melted under less extreme crustal conditions, and magma convection greatly increases the extent of partial melting in country rocks. Similar quantities of magma will produce, however, little melt in the upper crust (300° C).

If a substantial proportion of the zone of partial melting is liquid, then that liquid may mix with the magma. The amount of physical mixing between magma and country rock is partly dependent upon the relationship between the amount of time it takes the magma to solidify, and the time during which the country rock is melted. At the outer front of the zone of partial melting the country rock may still be melting after the mafic has cooled below its temperature of solidification. As long as the magma is above the solidus, however, the zone of partial melting in the country rock could mix with the magma and thus alter its composition. As an example, it can be assumed that solidification of the magma occurs at 900° C. Then an intrusive 6 km thick within country rock which had an original temperature of 500° C, will solidify after about 92,000 yr (Fig. 2). By that time the country rock would be partially melted out to 1.2 km from the contact, and most of the melt could mix with the magma before it solidified. If only a portion of the country rock melted, then limited amounts of granitic melt above and below the contact would be available for mixing. The refractory country rock material that does not melt may fall to the floor of the magma or be incorporated within it. In this manner the original magma could be diluted with both fused granitic material and refractory country rock.

The extent of partial melting around a slab shaped magma emplaced into a crust with a moderate geothermal gradient, is

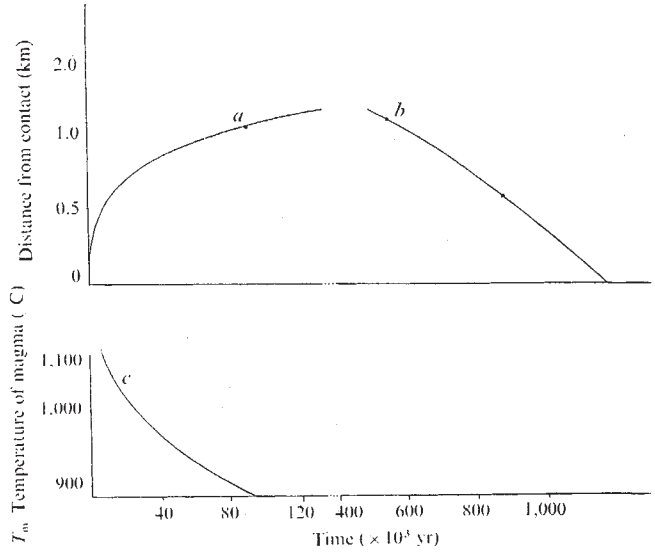

Fig. 2 *a*, The time which elapses between emplacement of magma and the onset of partial melting at increasing distances from the contact; *b*, the time which elapses between emplacement and the onset of solidification in the zone of partial melting at varying distances from the contact; *c*, the rate of cooling of a convecting magma.

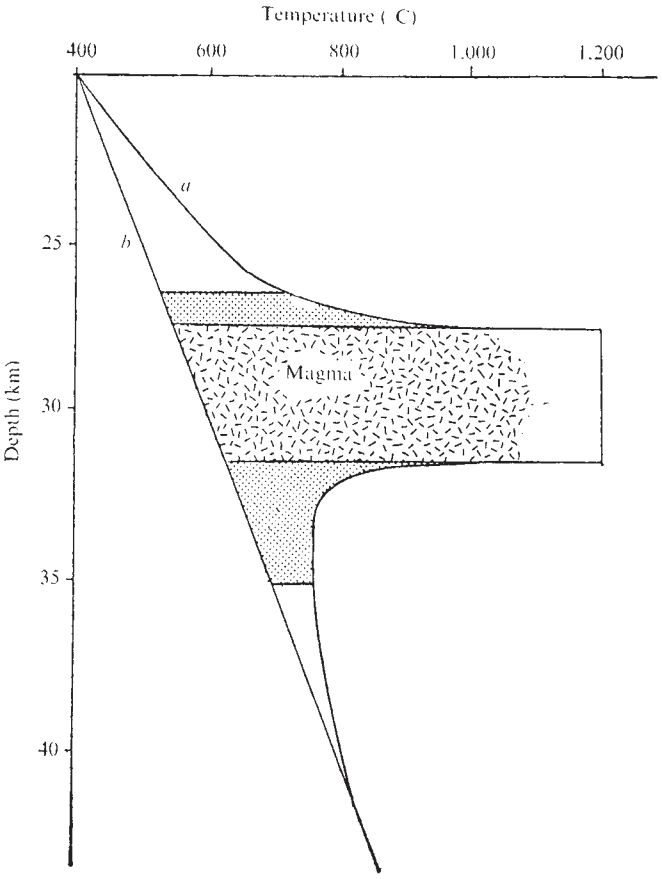

Fig. 3 Temperatures around an intrusive which is 4 km thick, and centred on a depth of 30 km: *a*, maximum temperatures; *b*, geothermal gradient; stippling, zone of partial melting. Initial temperature of magma = 1,200° C; *H*, = 50 calorie g^{-1}.

significantly different from that indicated by the model in Fig. 1. An asymmetrical orientation of the heat fluxing from the top and the bottom of the magma is produced. Taking an initial geothermal gradient of 20° C km^{-1}, and a magma slab 4 km thick emplaced at 30 km depth, the country rock temperatures below the magma would be at least 80° C more than above the magma, and those temperatures would increase with depth (Fig. 3). In this thermal model the values of the parameter given already define the initial boundary conditions, and crustal rocks initially hotter than 700° C (35 km depth) are assumed not to undergo partial melting. The maximum temperatures achieved in the country rock in a case such as that are shown in Fig. 3. About 1 km of country rock above the upper contact, and about 4 km below the magma, would undergo partial melting. The total width of the zone of partial melting would exceed the initial thickness of the magma. In that case, the total heat flux from the top of the magma would be about twice that from the bottom. As much as 5 km of the country rock would have been melted partially, whereas in the model shown in Fig. 1, the partially melted zone is only 1.6 km thick adjacent to a slab of magma of the same size, with initial country rock temperatures of 500° C.

The process of partial melting and solidification of the country rock adjacent to an intrusive, takes approximately 2×10^5 yr in the cases discussed. Solidification of the mafic parent rocks at 900° C takes about 10^4–10^5 yr. Thus, the zones of partial melting adjacent to a magma may have a higher mobility for a considerably longer period of time than either the solidified magma or the lower crustal material that has not undergone partial melting. These zones of partial melting may serve as sites for the emplacement of other material derived from the mantle, and further partial melting in the lower crust may follow.

These thermal models suggest that there are a number of possible ways in which an intermediate rock type could be derived from the mixing of a mantle derived parent with partially melted lower crustal rocks that are adjacent to the intrusion. Dense refractory material in the zone of partial melt, as well as in the parent magma, will tend to sink and lighter granitic fractions will tend to migrate upward. Granitic material may be formed from the partial melt zones above and below the magma and from differentiation of the original magma. These lighter granitic liquids could accumulate into large magmas in the lower crust and then migrate and collect to form plutons of batholithic proportions in the upper crust. In this two stage model of the origin of granitic batholiths along continental margins, the production of the intermediate granitic rocks may be intimately associated with the production and migration of magmas of andesitic to basaltic composition from active subduction zones at the continental margins.

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Thermal contraction joints in a spreading seafloor as origin of fracture zones

IF newly formed oceanic crust is moving away from a spreading axis, it can be expected to contract by cooling. Vertically, the cooling is expressed in the existence and cross-sectional shape of the mid ocean ridges, where there is a relationship between depth of the ocean and age of the crust[1]. A steady state solution for a model of a lithospheric plate which cools while moving[2–4] is compatible with data on topographical height.

Horizontally, in the direction of spreading, cooling will result in the formation of superficial horst and graben structures parallel to the median rift[5,6]. Also in the third direction—that of the ridge axis—internal stresses will arise which are much larger than the breaking strength of rock. If the thermal expansion coefficient λ, is 1.5×10^{-5} ° C^{-1}, and Young's modulus, *E*, is 1×10^6 kg cm^{-2}, a decrease in temperature of 1,000° C gives an internal tension of 15,000 kg cm^{-2}. Faulting can therefore be expected to occur perpendicular to the direction of the ridge axis, and I propose that fracture zones are the topographical expression of those faults (Fig. 1 and refs 7–10).

The hypothesis provides a model for an orthogonal median rift–fracture zone system[11]. The actual shape of the ridge as a whole will be dictated by other causes, such as the shape of the initial rift. That is exemplified by the parting of North America and South America from Africa. A change in spreading direction can also place constraints on the shape of the ridge. Straight ridges with fracture zones and no offset could be explained using this model:

the thermal contraction character is primary, and the transform fault function[13] is secondary.

Additional evidence of thermal contraction in the direction of the ridge axis comes from the distribution of fracture zones in the central North Atlantic and the equatorial Atlantic. There, the Mid-Atlantic Ridge is far from straight. The active sections of the fracture zones do not obey strictly the small circle pattern dictated by purely geometric or kinematic considerations[14]. The departures from that pattern are significant and apparently systematic (Fig. 2). Fracture zones and median rift segments still form an orthogonal system but the pattern as a whole becomes fanned, following to some degree the bends of the ridge.

The segments of the median rift and the fracture zones therefore form a complex dovetail construction, interlocking opposing lithospheric plates. Thus, in order to free the plates, about 6% of shortening in the direction of the ridge axis is needed. That is of the right order of magnitude compared with the maximum depth of the ocean, which in these parts varies between 5.5 and 6 km. That would lead to a total vertical contraction of 3–6%, depending on the thickness assumed for the lithosphere.

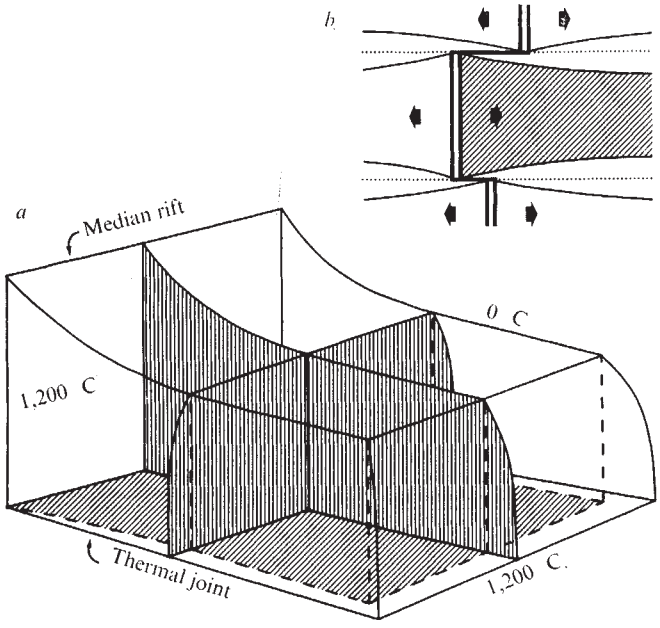

Fig. 1 *a*, Seafloor spreading implies cooling of newly formed lithosphere. A plate moving from the median rift will contract as shown. Thermal joints develop at right angles to the ridge axis. *b*, Plan view of thermal joints accommodating transform faults; arrows indicate spreading directions.

The fanning of fracture zones can be understood by considering the mechanism which makes the plates move. Thinking in terms of gravitational sliding, the analogy to glacier flow[15] may be extended by comparing the 'bights' of the Mid-Atlantic Ridge to a firn or névé: the shape of the bights would determine the flow pattern of the lithosphere which slides passively downwards to both sides, coagulating into rigid plates in which the stress trajectories are roughly parallel to the fossil fracture zones and to the growth lines of the lithosphere, that is, the magnetic lineations. The stress trajectories would then converge off the concave sides of the ridge, thus accounting for the fanning. A pull, exerted by the sinking lithosphere in the subduction zones and/or by a friction exerted by the upper mantle at the base of the lithosphere may be added to this. If the latter effect were dominant, however, fanning still occurs because the ridge represents a curvilinear zone of weakness in the lithosphere. A zone such as this (with a lower Young's modulus) in an elastic plate under tension would be reflected in the stress distribution. Experiments with a sheet of pararubber have shown that the longer axes of the strain ellipsoids are deflected towards the normal to the weakened zone.

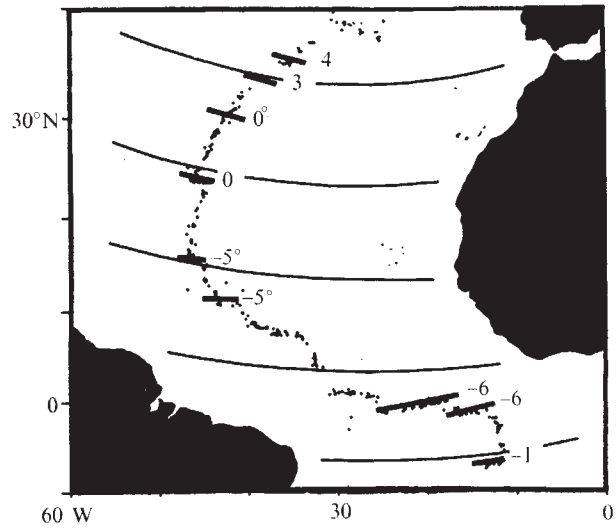

Fig. 2 Departure of fracture zone azimuths from a small circle pattern with a pole spreading at 63°N and 27°W.

Once the pattern has been established and the fracture zones begin to function as transform faults, the stress distribution will be affected if some form of welding occurs along the plane of the fracture zone. Extrapolation of the results of the experiments with pararubber, indicates that the larger principal stress may be expected to become deflected towards the normal to the fracture zone, thus releasing the normal stress in the plane of the fracture zone, and easing the movement.

The observation that fracture zones may be fanning implies that, although statistically and on a global scale the small circle concept[14,16] remains valid, poles derived from individual fracture zones or from a number of close fracture zones do not necessarily represent the true spreading pole. True spreading poles can only be found by averaging weighted azimuths of all of the fracture zones in a given area. The degree of fanning is also a function of age, with the fanning reaching its maximum at the ridge axis. The present data on the central North and equatorial Atlantic do not yet allow a final analysis.

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The TAG hydrothermal field

THERE is abundant evidence that seawater penetrates, and circulates within, the oceanic crust at active spreading centres. Low heat flow values from ridge crests imply that heat must be removed locally by water circulation[1-6]. Furthermore, hydrous metamorphosed oceanic crust and oceanic serpentines, which required voluminous quantities of water during their formation[7-9], also have isotopic compositions which indicate that the isotopic sources had low $\delta^{18}O$ values, such as are found in seawater[10]. The tectonic setting of strike-slip and normal faulting along the rift valley scarps of oceanic ridges is conducive to the penetration of dense, cold water down the fractures, the heating of the water at depth, and the exhalation of the less dense hydrothermal water as submarine springs along fracture systems.

During the 1972 and 1973 cruises of the Trans-Atlantic Geotraverse (TAG) project of the National Oceanic and Atmospheric Administration (NOAA), extensive bathymetric and geophysical surveys, and dredging, were carried out on an area two degrees square between the Kane and the Atlantis Fracture Zones on the Mid-Atlantic Ridge at 26° N. The usual assemblage of pillow basalts, metamorphosed pillow basalts, diabases and gabbros were dredged from scarps within 20 km of the distinct rift valley[11].

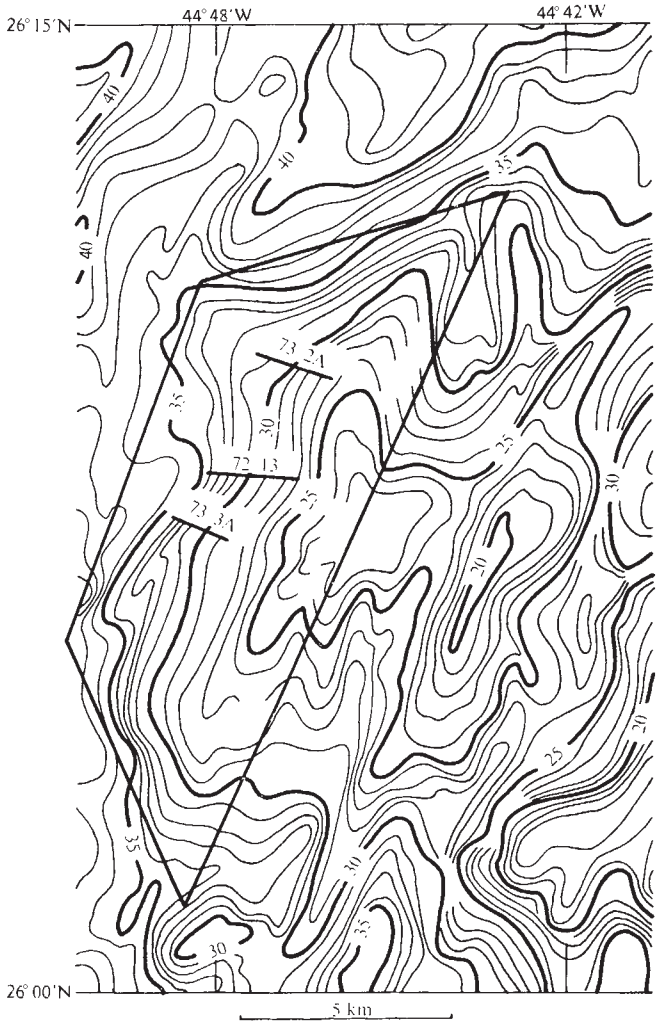

Fig. 1 Location of the 1972 and 1973 dredge sites from which hydrothermal manganese was recovered. This region covers part of the rift valley in the upper left corner, the eastern wall of the rift valley and a part of the eastern ridge crest highlands[15,35]. Contours are in hundreds of metres. The TAG hydrothermal field is shown within the solid straight lines which enclose an area of approximately 100 km².

Along one section of the eastern wall of the rift valley, however, every dredge recovered thick hydrothermal manganese encrustations that overlie and cement underlying talus (Fig. 1). Several lines of evidence suggest that this region is an active hydrothermal field.

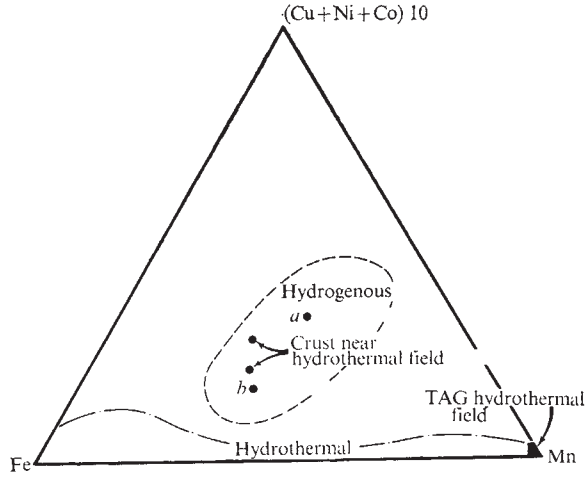

Fig. 2 Range of composition of manganese deposits from the TAG hydrothermal field on an Fe–Mn–(Cu+Ni+Co) 10 ternary diagram[16]. Average compositions of Pacific (*a*) and Atlantic (*b*) hydrogenous ferromanaganese nodule are from Price and Calvert[34]. The two hydrogenous ferromanganese crusts were collected from within 50 km of the hydrothermal field. Submarine, hydrothermal ferromanganese deposits have been reported from seamounts, submarine volcanoes, and the Afar and Red Sea spreading centres[16].

The manganese deposit is more than 50 mm thick but is found only 3–8 km from the median axis of the valley and thus must have grown about two orders of magnitude faster than typical open ocean, hydrogenous ferromanganese deposits. Rapid growth has been confirmed by $^{230}Th/^{234}U$ and $^{231}Pa/^{235}U$ data which indicate growth rates of 130–250 mm per 10^6 yr respectively[12]. The possibility that this rapid growth could be the result of a remobilisation of manganese in reduced, organic-rich, ponded sediment[13,14] is unlikely, because photographs of the ocean bottom[15] show the hydrothermal deposit growing on essentially bare, current swept talus 500–1,500 m above the rift valley floor. Chemically, the deposit is within the hydrothermal region (Fig. 2), and mineralogically it consists primarily of birnessite and secondarily of todorokite[12,17].

A positive thermal anomaly (0.14° C) was found in the water column over the hydrothermal region[18]. Also, the suspended weak-acid-soluble particles rich in iron and manganese, which were collected over the hydrothermal site, are far more concentrated than similar particles found to either side of the ridge crest[19]. Photographs show encrustations covering only the talus slopes[15]. Magnetic surveys indicate that the Brunhes normal axial anomaly contains a magnetic low region, the boundaries of which coincide closely with other estimations of the hydrothermal field boundaries[15,35] (Fig. 3). Presumably, the hydrothermal alteration of the magnetic mineral component of the pillow lavas has greatly reduced intensity of magnetisation[20]. Perhaps most significant, this observation of a distinctive magnetic irregularity associated with hydrothermal activity may become the geophysical exploration tool for the rapid location of new oceanic hydrothermal sites.

Ferromanganese deposits and ferromanganese-rich sediments at the interface of basalts and overlying sediments are common features of ophiolites[21-23]. Petrological evidence suggests that some ophiolites may have been formed off

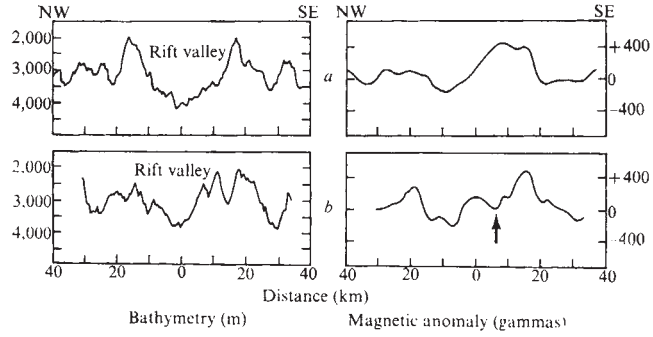

Fig. 3 Bathymetric and residual magnetic profiles across the rift valley of the Mid-Atlantic Ridge near 26°N (refs 15 and 35). *a*, 12 km north of the TAG hydrothermal field, showing the strong Brunhes normal over the axial valley, which is typical of all of our profiles from outside the hydrothermal field; *b*, profiles across the TAG hydrothermal field; the arrow indicates the magnetic low in the axial anomaly.

ridge axes[24-26], suggesting that hydrothermal mineralisation could occur away from a ridge axis. In any case, this does not detract from the probability that the hydrothermal mechanisms that operated in many ophiolitic complexes to form cupreous pyrite bodies[23,27-29], altered pillow lavas, and overlying manganiferous oxides, were similar to that now operating on the Mid-Atlantic Ridge at 26°N. Although massive sulphide bodies have not yet been recovered from this site, disseminated pyrite occurs in greenstone pillow lavas dredged from adjacent fault scarps. Sulphide deposits are not rare in altered oceanic rocks[30,31]. Thus, we infer that the TAG hydrothermal field may at present be forming Troodos-type, massive, stratiform, sulphide bodies within pillow lavas below the manganese deposits.

The sharp transition from the precipitation of metal sulphides to the precipitation of metal oxides at the seawater–basalt interface is probably controlled largely by a sharp gradient of oxygen concentration from hydrothermal fluids which have little oxygen, to bottom waters which have relatively high concentrations of oxygen. A reasonable estimate of the fugacities of oxygen, and sulphur, and of the *p*H values that may operate in such a system, has been given by Meyer and Hemley[32]. The source of sulphur in these hydrothermal systems may in part be reduced sulphate from the influxed seawater, but some sulphur is probably leached from rocks that react with hydrothermal fluids (unpublished work: fresh pillow lavas, 1,000 p.p.m. sulphur; adjacent greenstones, 400 p.p.m. sulphur). The iron and manganese are probably leached from crustal rocks under low fO_2 conditions; much of the dissolved iron may be removed from fluids as sulphides at lower temperatures close to the surface, but a significant quantity becomes suspended in the ocean water column[19]. Because the iron readily precipitates as hydroxides at lower oxygen concentrations than manganese compounds[33], the particulate iron phase must bypass the manganese crust at the TAG hydrothermal field.

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Ancient tropical weathering in Calabria

FROM the lower Crati valley in Calabria, to the Peloritani Mountains in Sicily, metamorphic and intrusive igneous lithotypes represent the bulk of the Apennine mountain range. A wide cover of Tertiary sediment overlies these rocks, mainly along the coastal areas. The metamorphic and igneous rocks are in a weathered state. The weathering may be tens or even hundreds of metres deep. Deep weather-

ing characterises crystalline rocks in a rapidly uplifting region such as Calabria.

Current opinion is that the weathering of the intensely fractured crystalline rock masses has resulted from the action of Mediterranean climatic conditions: short rainy winters, alternating with long dry summers, and periods of frost[1].

In fact today the area has a meso-Mediterranean type of climate[2]: warm dry summers, relatively mild and humid winters and mostly clear, sunny weather throughout the year. Rainfall varies from 600 mm yr^{-1} to 1,500 mm yr^{-1}, with about 45% falling during the winter and only 5–8% falling in the summer period, and there is considerable variation from year to year. The mean annual temperature is about 17° to 18° C along the coast, about 14° C in the transition zone to the mountains and lower in the highland area[3].

I have collected evidence which suggests that deep weathering is peculiar to tropical rain-forest areas, which have mean annual temperatures between 20° C and 30° C, little annual temperature range and no frost. In these areas rainfall may be over 3,000 mm yr^{-1} (ref. 4).

In present day and ancient rain-forest tropical areas typical profiles of weathering of crystalline rocks, such as granite or diorite, can be seen. Organic sandy soil overlies decomposed rocks of a sandy texture within which the original discontinuities are still preserved (saprolite). The layer is underlain by spheroidally weathered boulders, generally surrounded by sandy material and at the bottom of the profile there are jointed, deeply weathered rocks with sand or clay along the major joints[5].

This is the weathering profile commonly observed in Calabrian granites.

Furthermore, there are several conditions, in addition to the presence of a favourable climate, which must be fulfilled before such weathering can occur. These include a peneplained landscape, prolonged tectonic quiescence, only a slight amount of erosion, and fracturing to a great depth[6]. Only the last of these conditions has been fulfilled in Calabria during the recent geological past, and it is the only condition which is satisfied there today.

It thus seems probable that what occurs in Calabria is the intense erosion of an exposed, fossil, deeply weathered mantle. This implies that evidence of the necessary conditions will only be found in older geological formations.

The deep weathering of Calabrian crystalline rocks is normally considered as an inconsistency which disturbs petrological and structural observations. What I have suggested provides the opportunity to understand the tectonic events which occurred since the production of the weathering.

Moreover, the need of a better definition of the real relationships between fossil weathered zones and present day morphological features and erosional processes, cannot be disregarded when considering problems of soil erosion and natural slope stability.

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Mechanism for producing NO from nitrogen in flames

THE principal chemical reactions which generate the pollutant nitric oxide, NO, from atmospheric nitrogen in combustion systems, are generally recognised[1] to be those first proposed by Zeldovich[2]:

$$O + N_2 \rightarrow NO + N \quad (1)$$
$$N + O_2 \rightarrow NO + O \quad (2)$$

It is the first reaction in this chain sequence which determines the rate of reaction, and therefore under some combustion conditions reaction (2) may be supplemented by others, such as $N + OH \rightarrow NO + H$. The Zeldovich mechanism is thought[1] to operate generally in flames where oxygen atoms are first produced in the reaction zone, often in concentrations well above those of equilibrium at the temperature which the burnt gases finally attain[3]. Measurements[1,4,5] of the rate of production of NO in flames, have revealed a consequent acceleration arising from these superequilibrium concentrations of oxygen atoms.

In contrast, Fenimore[6] concluded that NO is produced by an additional mechanism. This is thought to operate in or very close to the reaction zone of a flame, and involves a hydrocarbon fragment which attacks molecular nitrogen; for example, $CH + N_2 \rightarrow HCN + N$. Nitric oxide could then be produced by reaction (2), as well as by the oxidation of HCN. It should be noted that hydrocarbon radicals such as CH are found generally only in or close to the reaction zone of a flame, because they disappear in the burnt gases downstream by rapid bimolecular processes for example, by $CH + O \rightarrow CO + H$. Because flame gases at atmospheric pressure have a residence time in their reaction zone of no more than 50 μs, the outcome has accordingly been termed 'prompt' NO. Although Fenimore's suggestion has been rejected several times[1], particularly in systems which are fuel lean, it has received some support[7,8].

The work reported here represents a preliminary attempt to test whether 'prompt' NO is produced in addition to that from the Zeldovich scheme. A fuel-rich flame, of unburnt composition $H_2/O_2/N_2 = 3/1/4.75$, was burnt at atmospheric pressure on a water-cooled burner made from stainless steel hypodermic tubes. The design of the burner was similar to that described by Padley and Sugden[9], except that it produced only one flame of 16 mm diameter, approximately 0.3 m long, with a flat reaction zone, about 0.1 mm thick, located about 1 mm from the burner face. The burnt gases of the flame had a temperature of 2,000 K, which corresponds to an equilibrium concentration for NO of around 7 parts per million (p.p.m.). The gases at various points along the axis of this flame were sampled through a water-cooled probe (made entirely from quartz) into a chemiluminescent NO analyser. That instrument gave the concentration of nitric oxide as a function of axial distance along the flame. As the radial profiles of velocity, temperature and concentration in the early part of the burnt gases are 'top hat' ones, the system is a good approximation to a one-dimensional flow system. Determinations of concentrations, such as [NO], as a function of axial distance, can easily be transformed into functions of time for the first 50 mm of the burnt gases, where the axial velocity is constant, at 11.3 m s^{-1}, and the gases are not disturbed by air entrainment or after-burning. Such measurements were made in the flame itself and also with a little of the hydrogen replaced with

acetylene to give an overall concentration of 1% by volume of hydrocarbon. Such an amount of acetylene in the unburnt gases has been shown[10] not to affect the temperature or composition of the burnt gases, apart from the introduction into them of a small amount of CO. Acetylene is, however, likely to release many short-lived radicals into the reaction zone, such as CH and C_2.

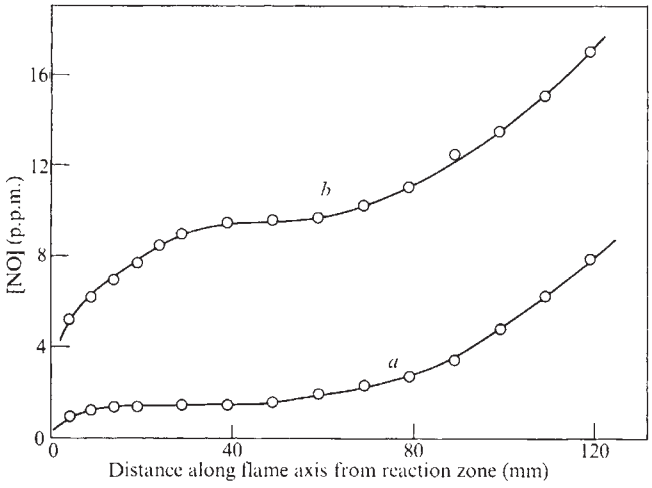

Fig. 1 Variation of the concentration of nitric oxide with position along the axis of a flame of unburnt composition $H_2/O_2/N_2$ = 3/1/4.75, and temperature 2,000 K. *a*, [NO] without additives to the flame; *b*, burner supplies contain 1% by volume of acetylene.

The results are shown in Fig. 1. In the absence of added acetylene, [NO] rises sharply in the first 5 mm of the burnt gases and is then almost constant up to about 50 mm (Fig. 1*a*). After that, there is another definite increase probably because of the entrainment of O_2 and N_2 from the surrounding atmosphere, and after-burning of the excess hydrogen, with a consequent temperature rise. The steady level attained by [NO] around 50 mm downstream, is roughly 0.2 of that expected for equilibrium. It is thus clear that the formation of NO, which in this instance must be by the Zeldovich scheme, is slow under these particular conditions, in spite of the fact that oxygen atom concentrations are expected[9,10] to exceed their equilibrium values by up to a factor of 3×10^3.

In the presence of 1% by volume of acetylene, [NO] rises to a first steady level (at around 40–60 mm) almost seven times higher (Fig. 1*b*), which is approximately that for equilibrium. Subsequently, [NO] increases downstream because of air entrainment and the gradual temperature increase with after-burning; after the first 40 mm the trends of the two mixtures are parallel (Figs 1*a*, *b*). These observations can be interpreted only in terms of two additive mechanisms for producing NO. The Zeldovich scheme is 'singled out' by the first mixture (Fig. 1*a*), relating entirely to the simple $H_2 - O_2 - N_2$ system. Added to curve *a* is that amount of NO produced in the early part of the flame (although here it does not seem to be confined entirely to the reaction zone but extends to the first 40 mm of the burnt gases) giving curve *b* on the addition of 1% of acetylene. This behaviour, which arises from the presence of hydrocarbon, corresponds to the production of 'prompt' NO. These data relate to the particular flame conditions used here, and although they demonstrate that, if after-burning is neglected, the 'prompt' NO is almost six times that from the Zeldovich scheme, that is not always so. In fact, the ratio of 'prompt' to Zeldovich NO depends on such variables as $[H_2]/[O_2]$ in the unburnt gas supplies, flame temperature, the quantity of acetylene added, and so on. These matters will be discussed elsewhere.

Although these observations establish the importance of 'prompt' NO under these conditions, the chemical reaction initially responsible has not been identified. We give some of the possibilities in Table 1, together with their associated heats of reaction, for all species in their ground electronic states. These enthalpy changes were derived from dissociation energies of diatomic molecules quoted by Gaydon[11] and of polyatomic molecules listed by Herzberg[12], Cottrell[13] or the JANAF Tables[14]. Five reactions (5, 7, 15, 16 and 17 in Table 1) seem to be the only candidates for the attack of N_2 by a hydrocarbon fragment, because they have the lowest heats of reaction. Admittedly, activation energies can exceed endothermicities, and also the various hydrocarbon radicals do have differing concentrations in a flame. But as an increase of 100 KJ mol^{-1} at 2,000 K in the activation energy of a reaction reduces its rate by a factor of 400, we conclude that all the reactions (Table 1), apart from the five mentioned, can be excluded on thermochemical grounds. Of the five possibilities, reactions (16) and (17) are unlikely, because they involve a four-centre reaction. In the same way, reaction (15) is a very weak candidate, because its transition state lacks plausibility; furthermore, CH_3O is not one of the most common radicals associated with the combustion of a hydrocarbon. That leaves reactions (5) and (7). The latter is more likely both sterically and energetically; the primary products of the attack of N_2 are HCN and N. If reaction (5) also operates, only one other product, NH, is involved, which is known[15] to have its concentration in these flames linked to that of free nitrogen atoms:

Table 1 Possible reactions for attacking N_2 in flames

No.	Reaction	Standard enthalpy changes at 0 K ΔH J mol^{-1}
(3)	$CH_3 + N_2 \rightarrow HCN + NH_2$	157±22
(4)	$CH_3 + N_2 \rightarrow CN + NH_3$	244±12
(5)	$CH_2 + N_2 \rightarrow HCN + NH$	88±30
(6)	$CH_2 + N_2 \rightarrow CN + NH_2$	222±30
(7)	$CH + N_2 \rightarrow HCN + N$	9±15
(8)	$CH + N_2 \rightarrow CN + NH$	200±25
(9)	$C + N_2 \rightarrow CN + N$	193±17
(10)	$CO + N_2 \rightarrow CN + NO$	636±11
(11)	$HCO + N_2 \rightarrow HCN + NO$	240±21
(12)	$HCO + N_2 \rightarrow CN + HNO$	560±40
(13)	$H_2CO + N_2 \rightarrow HCN + HNO$	350±31
(14)	$H_2CO + N_2 \rightarrow HNCO + NH$	337±40
(15)	$CH_3O + N_2 \rightarrow HNCO + NH_2$	58±63
(16)	$C_2 + N_2 \rightarrow CN + CN$	48±25
(17)	$C_2H + N_2 \rightarrow HCN + CN$	80±48

$$NH + OH \rightleftharpoons N + H_2O \quad (18)$$

There is thus very little to distinguish between the products of reactions (5) and (7). In any event, whether NH or N is produced initially, NO is most probably formed[15] by:

$$N + OH \rightleftharpoons NO + H \quad (19)$$

in these hydrogen-rich flames.

The other product, HCN, is common to both reactions (5) and (7), as well as to other reactions in Table 1, on account of its stability. Its concentration is coupled[16] to that of CN by an equilibration of

$$HCN + H \rightleftharpoons CN + H_2 \quad (20)$$

with NO resulting[16] from one of two exothermic reactions:

$$CN + O_2 \rightarrow CO + NO \quad (21)$$
$$CN + OH \rightarrow CO + NH \quad (22)$$

Reaction (22) is followed by reaction (18) and then by reaction (19). In a fuel-rich flame, such as that studied here, $[O_2] \ll [OH]$ and reaction (21) is, therefore, probably unimportant.

These considerations indicate that in fuel-rich systems NO can be formed from HCN by a succession of four reactions: reactions (20), (22), (18) and (19), whereby HCN → CN → NH → N → NO. Taking reaction (7), the most probably primary

reaction, HCN and N will be produced. Both of these will produce NO, but HCN—at the beginning of the sequence of reactions—will do so at a much slower rate than N atoms, which give NO in a rapid, single-step process. It could thus well be that roughly half of the NO is generated very quickly, and the rest relatively slowly from HCN, with the result that the appearance of 'prompt' NO could extend into the burnt gases, as observed in Fig. 1. In this context, HCN has been observed[7] in the burnt gases of hydrocarbon flames as an intermediate in the production of 'prompt' NO.

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Evidence for a complete mixed valence solid solution series in $Fe_3^{2+}(H_2O)_3[PO_4]_2$ (phosphoferrite) $Fe_3^{3+}(OH)_3[PO_4]_2$ (kryzhanovskite)

HOMONUCLEAR intervalence-transfer absorption of the first transition series cations has aroused considerable interest for two reasons[1]. First, it may give rise to intense colour which is not predicted by the addition of the colours of either of the individual ions. Second, it may lead in some chain structures to strong anisotropy in electronic conduction. A classic example is vivianite, $Fe_3^{2+}(H_2O)_8[PO_4]_2$: although colourless when fresh, upon partial oxidation of the transition metal the compound assumes a deep blue which is at its maximum intensity when the electric vector of polarised light is parallel to the axis of the two iron atoms in the octahedral edge-sharing dimer of the crystal structure. Extensive study on this and on other systems has led to a considerable body of literature[1].

In the homologous series $Fe_3^{2+}(H_2O)_n[PO_4]_2$, where all n water molecules and all oxygens associated with the phosphate tetrahedra are also bound to the inner sphere of the transition metal, extensive oxidation of $n = 8$ (vivianite) and $n = 4$ (ludlamite), leads to destruction of the crystal structure[2]. For $n = 3$ (phosphoferrite), it is possible to synthesise the crystalline ferric analogue of a natural mineral with initial composition $(Fe_{2.1}^{2+}Mn_{0.8}^{2+}Ca_{0.1}^{2+})(H_2O)_3[PO_4]_2$ by heating crystals in air[2]. These observations suggest that the balance of electrostatic valence is the basis for the stability of the $n = 3$ homologue over an extensive range of Fe^{2+} oxidation to Fe^{3+}.

I have examined single crystals of pure synthetic $Fe_3^{2+}(H_2O)_3[PO_4]_2$ with the phosphoferrite structure. The use of the pure compound makes it possible to study the complete Fe^{2+}–Fe^{3+} mixed valence series without ambiguities which might arise from other substituents such as Mn^{2+} and Ca^{2+} usually found in natural crystals. Crystals of the synthetic compound are pale green with prismatic development parallel to the [100] axis. (Fig. 1).

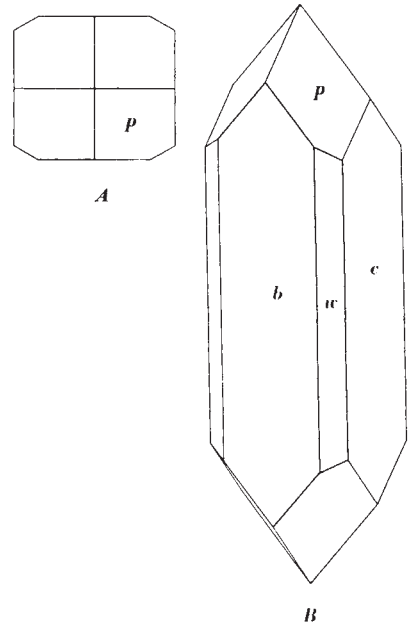

Fig. 1 Crystal development of $Fe_3(H_2O)_3[PO_4]_2$ (phosphoferrite) showing the forms $c\{001\}$, $b\{010\}$, $w\{021\}$, and $p\{111\}$. *A*, Plan, *B*, clinographic projection.

Several single crystals were placed on a glass slide and heated in a muffle furnace at 180° C for 24 h. The products were deep red and optically transparent with no evidence of decomposition. In the early stages of heating, the crystals became progressively darker green. Crystals of the ferrous compound were crushed, powdered with Si (a = 5.4301 Å) standard and the mixture uniformly smeared on a glass slide. A chart diffractogram with scan speed of 1° min^{-1} in 2θ utilised graphite monochromatised CuKα radiation. The same slide was then heated at 180° C for 24 h and another diffractogram was prepared. Rotation and Weissenberg photographs were obtained for the ferrous compound and for the same crystal heated at 180° C for 24 h. The space group for both crystals was established as *Pbna*.

Results of the powder studies, the calculated data obtained by least-squares refinement, are given in Table 1 and the single crystal studies in Table 2. Evidence for the existence of the ferric end member is provided by the deep red colour of the crystal, the volume reduction of about 6.1 $Å^3$ per Fe atom computed from the cell volume, and from the optical properties of the two compounds. The indices of refraction are α 1.678(3), β 1.695(3), γ 1.710(8) with Z || a, sign (—), 2V large for the ferrous compound and α 1.873(3), β 1.878(3), γ 1.995(4) with Y || a, sign (+), 2V ~ 20° for the ferric equivalent. Gladstone–Dale calculations provide densities which are similar to those computed from the unit cell. I did not determine specific gravity directly because of the possibility of cracks in the heated compound.

The evidence clearly points to a complete solid solution series between $Fe_3^{2+}(H_2O)_3[PO_4]_2$ (phosphoferrite) and

Table 1 X-ray powder data for $Fe_3^{3+}(H_2O)_3[PO_4]_2$ and $Fe_3^{3+}(OH)_3[PO_4]_2$

Phosphoferrite $Fe_3^{2+}(H_2O)_3[PO_4]_2$				Kryzhanovskite $Fe_3^{3+}(OH)_3[PO_4]_2$			
I/Io	*d*(obs)	*d*(calc)	*hkl*	*I/Io*	*d*(obs)	*d*(calc)	*hkl*
15	5.379	5.375	111	20	5.189	5.185	111
70	4.997	4.996	020	60	4.867	4.869	020
40	4.722	4.721	200	50	4.751	4.753	200
10	4.320	4.325	021	25	4.269	4.271	210
35	4.272	4.268	210	10	4.010	4.006	022
12	3.931	3.932	121	20	3.812	3.812	121
15	3.431	3.431	220	12	3.399	3.401	220
100	3.190	3.189	221	100	3.128	3.131	221
25	3.087	3.089	122	35	3.064	3.063	202
10	2.958	2.957	301	40	2.942	2.942	122
15	2.757	2.756	103	20	2.679	2.681	230
50	2.721	2.721	230	10	2.591	2.593	222
5	2.687	2.687	222	15	2.573	2.571	103
30	2.656	2.657	113	5	2.523	2.521	321
10	2.495	2.496	023	25	2.489	2.486	113
10	2.465	2.465	312	5	2.436	2.435	040
25	2.413	2.413	123	10	2.407	2.408	312
10	2.327	2.326	141	10	2.342	2.342	023
10	2.218	2.220	411	5	2.329	2.329	041
15	2.212	2.211	331	10	2.271	2.274	123
15	2.160	2.163	042	10	2.262	2.264	213
15	2.108	2.108	142	10	2.220	2.218	411
5	1.9560	1.9556	323	10	2.182	2.182	331
10	1.6109	1.6112	161	10	2.080	2.081	042
20	1.5720	1.5715	352	15	1.9727	1.9733	332
10	1.5449	1.5444	244	15	1.8836	1.8845	422
				10	1.8785	1.8768	341

$Fe_3^{3+}(OH)_3[PO_4]_2$ (kryzhanovskite). In addition, the mosaic spreads of the same reflections for the two compounds were identical within error of the powder diffractometer experiment. This suggests that the oxidised equivalent is a single homogeneous crystal. One interesting aspect of this solid solution series which involves a ferrous and a ferric end member is that no vacancies need occur over the octahedral sites to maintain charge balance. Moore[2] has proposed that the intracrystalline reaction is an auto-oxidation–reduction, that is, $2[Fe^{2+}(H_2O)^0] \rightarrow 2[Fe^{3+}(OH)^-] + H_2\uparrow$.

The interesting feature of the phosphoferrite structure includes a kinked octahedral edge-sharing chain which is oriented parallel to the [001] axis of the crystal[2]. Equivalent chains are linked to each other only through the corners with Fe-Fe distances considerably longer than the distances within the chains. Writing the cell formula $Fe(1)_4Fe(2)_8(H_2O)_{12}[PO_4]_8$ to distinguish the nonequivalent Fe atoms, the sequence of iron atoms in the chain is ... Fe(1)–Fe(2)–Fe(2)–Fe(1)–Fe(2)–Fe(2) ... In the natural type kryzhanovskite with octahedral composition ($Fe_{1.8}^{3+}Mn_{1.0}^{2+}Ca_{0.1}Mg_{0.1}$), the ordering scheme is nearly $Fe(1) = Fe^{3+}$ and $Fe(2) = Mn_{1.0}^{2+}Fe_{0.8}^{3+}Ca_{0.1}Mg_{0.1}$, according to the structure analysis of Moore[2]. It is not known, however, if the Fe(1) site or the Fe(2) site oxidises first or if the oxidation sequence is disordered for intermediate mixed valences of the pure iron compound.

Several crucial experiments are in progress including routine refinement of the crystal structures of the ferrous and ferric end members and refinement of the intermediate composition $Fe^{3+}/(Fe^{2+} + Fe^{3+}) = 0.33$. This last experiment is particularly important since it should answer the question whether the ordering scheme is $Fe(1) = Fe^{3+}$, $Fe(2) = Fe^{2+}$; $Fe(1) = Fe^{2+}$, $Fe(2) = 1/2Fe^{2+} + 1/2Fe^{3+}$; or $Fe(1) = Fe(2) = 2/3Fe^{2+} + 1/3Fe^{3+}$. In addition, single crystals of a range of compositions can be conveniently oriented for optical study.

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Table 2 Crystal cell parameters for $F_3^{2+}(H_2O)_3[PO_4]_2$ and $Fe_3^{3+}(OH)_3[PO_4]_2$

	Phosphoferrite $Fe_3^{2+}(H_2O)_3[PO_4]_2$	Kryzhanovskite $Fe_3^{3+}(OH)_3[PO_4]_2$
a(Å)	9.442(5)	9.505(6)
b(Å)	9.992(4)	9.739(6)
c(Å)	8.644(4)	8.012(5)
V(Å³)	815.5(1.1)	742.0(1.1)
Z	4	4
ρ (calc)* gm cm⁻³	3.35	3.66
ρ (calc)† gm cm⁻³	3.34	3.71

* From the cell volume, formula weight and Avogadro's number.
† From the Gladstone–Dale relationship. The specific refractive energy for Fe_2O_3 was adopted as $k = 0.272$ from Moore[3] for epidotes ($k = 0.270$) and from strengite and metastrengite, $Fe^{3+}(H_2O)_2[PO_4]$, ($k = 0.274$). The specific refractive energies for the other more accurately known oxides are from Larsen and Berman.[4]

Viscoelastic effects in boundary lubrication

THE traditional 'time dependence of static friction' approach, which is used to explain observed variations in static friction, has limited validity[1]. The appropriate governing variable is the rate of increase of the tangential force coefficient, $\dot{\theta}$, and not the static contact time as has been suggested previously[1]. ($\dot{\theta} = \dot{F}/N$ where N is the normal load forcing opposing surfaces together and $\dot{F}$ is the rate of application of the shearing force.)

Experimental work using steel surfaces lubricated by various surface films has shown that surface conditions play a very large role in determining the exact μ_s–$\dot{\theta}$ relationship for a given friction couple where μ_s is the coefficient of static friction. The results are briefly summarised in Fig. 1. The static friction

of steel surfaces, tested when coated with thin oxide films or under 'clean' (high vacuum) surface conditions, was not dependent upon $\dot{\theta}$ over the given range of $\dot{\theta}$ values. When the surfaces were covered with a monolayer of a metallorganic soap, however, the μ_s–$\dot{\theta}$ behaviour exhibited the expected dependence on rate. The shape of the μ_s–$\dot{\theta}$ curve (Fig. 1) depends on the boundary lubricant involved, but all three curves show an upper and a lower asymptote. Exposing the steel surfaces to the laboratory atmosphere results in a similar μ_s–$\dot{\theta}$ behaviour, presumably because of the adsorption of hydrocarbons on the surfaces. This experimental work demonstrates clearly that the static friction of steel can be significantly modified by appropriate boundary lubricants.

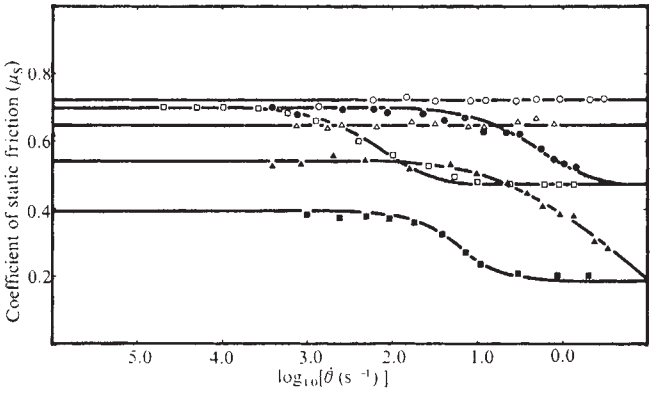

Fig. 1 Static friction, μ_S against $\log_{10}\dot{\theta}$ for C1020 steel with various surface treatments. △, Water-formed oxide, 0.2×10^{-8} torr; exposed to $Ca(OH)_2$ in air for 20–48 h—Langmuir Blodgett deposition; □, $Ca(St)_2$—Langmuir Blodgett deposition; ■, $Fe(St)_3$—abrasion technique of Smith and McGill[2].

A model representing the coefficient of static friction, μ_s, as a function of $\dot{\theta}$ has been developed. This model is based on the assumption that the real contact area between the surfaces grows in a viscoelastic manner until a critical shear stress is reached. The model has been formulated (see Fig. 2) by considering that the interfacial contact region deforms as a three-parameter viscoelastic solid having k_1 and k_2 as its elastic parameters, and c as the viscosity parameter. The initial area of contact, A_i, is established by N: $A_i = N/p_m$, where p_m is the mean yield pressure of the material. Junction growth ends when a critical shear stress, T_0, is reached. The μ_s–$\dot{\theta}$ curve then has the form:

$$-\frac{k_1}{p_m} + \left[\frac{k_2}{(k_1 + k_2)}\right]\tau\dot{\theta}\left[1 - \exp\left(-\frac{\mu_s}{\dot{\theta}\tau}\right)\right] + \left[\frac{(k_1}{T_0)} - 1\right]\mu_s = 0 \quad (1)$$

where $\tau = c\left[\frac{(k_1 + k_2)}{k_1 k_2}\right]$ is the relaxation time.

This equation predicts upper and lower asymptotes for μ_s as follows:

$$\mu_{s\,max} = \frac{T_0}{p_m}\left[\frac{k_1}{(k_1 - T_0)}\right] \text{ for } \dot{\theta} \ll 1 \quad (2)$$

$$\mu_{s\,min} = \frac{T_0}{p_m}\left[\frac{(k_1 + k_2)}{(k_1 + k_2 - T_0)}\right] \text{ for } \dot{\theta} \gg 1 \quad (3)$$

Using the experimental data and these equations, a relaxation time may be assigned to each of the boundary lubricants. For given asymptotes, a larger relaxation time shifts the μ_s–$\dot{\theta}$ curve to the left. This shift may be interpreted as an improvement in the lubrication of the surfaces.

In addition, from the experimentally determined relaxation times, a dimensionless μ_s–τ^* curve was prepared for all the boundary lubricants which had the same upper and lower asymptotes. All the experimental data fall on to this general curve.

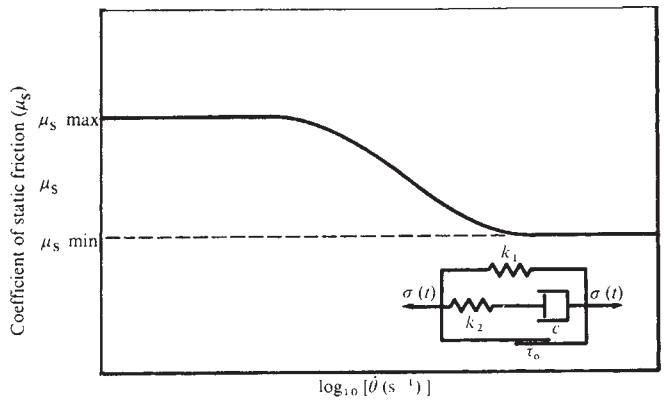

Fig. 2 General form of the μ_S–$\dot{\theta}$ curve.

In a further study, the effect of temperature on the μ_s–$\dot{\theta}$ behaviour of steel lubricated with a calcium stearate monolayer showed that the relaxation time decreased with increasing temperature. These results demonstrated that an increase in temperature produced a progressive deterioration in the quality of lubrication in the context of the shift effect discussed earlier.

From equation (1) it is obvious that a 'loading time', where F is constantly increasing, and a 'delay time', where F is held constant, are not equivalent in their effect on area growth. For the calcium stearate monolayer case, for example, it was calculated that for a delay time of 1 s, the contact area is increased by 0.5%. Even if the delay time is infinite, the area increase is only about 10%. These calculations reveal why delay times will not increase significantly μ_s values, and why measurements of the interfacial electrical resistance[1] do not indicate appreciable area growth during the delay period.

Thus, the transverse load rate dependence of static friction may be associated with the viscoelastic properties of lubricant monolayers. It is believed that the viscoelastic explanation of the observed friction behaviour reflects more accurately the physical processes involved in the sliding of one body over another.

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An auditory illusion

I HERE report a novel and striking auditory illusion, which provides a paradox for theories of pitch perception and auditory localisation[1,2]; and which varies in correlation with the handedness of the listener. The stimulus configuration which produced the illusion consisted of a sequence of tones, alternating in pitch between 400 Hz and 800 Hz (Fig. 1*a*). Each tone lasted 250 ms, with no gap between tones. The sequence was presented at equal amplitude to both ears simultaneously; however, when one ear received 400 Hz the other received 800 Hz, and *vice versa*. Thus the same two-tone combination was presented constantly, but the ear of input for each component switched every 250 ms.

It is easy to imagine how this pattern should sound if perceived correctly. However, 86 subjects listened to a 20-s presentation of this sequence, and none obtained the correct percept. Instead, various illusory percepts were reported (Table 1), the most common being a single tone oscillating

Table 1 Categories of illusory percept

	Octave (%)	Single pitch (%)	Complex (%)
Right handers ($n = 53$)	58	25	17
Left handers ($n = 33$)	52	9	39

Figures show the percentages of right or left-handed subjects obtaining a given percept. The two groups of subjects differed significantly in the relative distribution of their percepts ($\chi^2 = 6.8$, d.f. = 2, $P < 0.05$). Octave indicates the illusion of a single tone oscillating from ear to ear, whose pitch oscillates synchronously from one octave to another. Single pitch indicates the illusion of a single tone oscillating from ear to ear, whose pitch either remains constant or shifts very slightly. Complex comprises various complex percepts, such as two alternating pitches in one ear, with a third pitch intermittently in the other; or two alternating pitches oscillating from ear to ear and two further pitches alternating at twice the speed localised at the back of the head. This group of percepts tends to be quite idiosyncratic and unstable; however, no subject reported the stimulus correctly.

from ear to ear, whose pitch also oscillated from one octave to the other in synchrony with the localisation shift (Fig. 1*b*). Two subjects with absolute pitch identified these oscillating pitches as G_4 (392 Hz) and G_5 (784 Hz); these are closest on the musical scale to the 400 Hz and 800 Hz presented. The percept depicted by one of these subjects is shown on Fig. 2.

This synchronous alternation of apparent pitch and localisation is paradoxical. The perception of alternating pitches can be explained by assuming that the listener processes the input to one ear and ignores the other. But then both the alternating pitches should seem to be localised in the same ear. Alternatively, the oscillation of a single tone from ear to ear can be explained by supposing that the listener suppresses the input to each ear in turn. But then the pitch of this tone should not change with a change in its apparent localisation.

A further surprise involves the patterns of apparent localisation for the two pitches at the two ears in right and left-handed subjects. Right handers had a highly significant tendency to hear the high tone in the right ear and the low tone in the left ($P < 0.001$, two-tailed on a binomial test), and also to maintain a given localisation pattern when the placement of the earphones was reversed ($P < 0.001$, two-tailed on a binomial test). Left handers did not localise the tones preferentially either way, however, though they showed a marginally significant

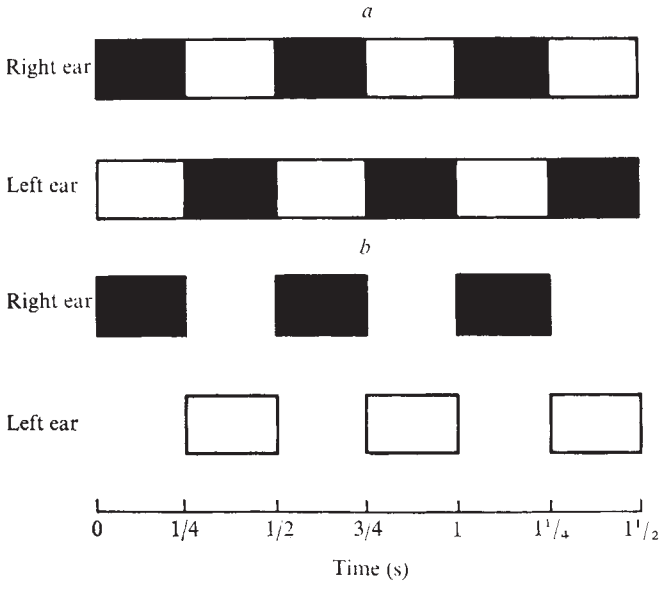

Fig. 1 *a*, Representation of the stimulus configuration which produces the illusion. Filled boxes represent tones of 800 Hz and unfilled boxes tones to 400 Hz. The tones were sinusoids, and their phase relationship varied randomly. They were played to subjects at a level of 75 dB SPL. *b*, Representation of the illusory percept most commonly obtained.

tendency to retain a given localisation pattern when the earphones were reversed ($P = 0.05$, two-tailed on a binomial test). This difference between right and left handers is consistent with findings relating patterns of hemispheric dominance to handedness[3,4] and so suggests that we tend to localise the high tone to the side producing the most effective input to the dominant hemisphere[5-7] and the low tone to the other side.

The localisation patterns on Table 2 are based on the two initial presentations of the sequence; however, with continued listening these patterns sometimes reverse, as in reversals of ambiguous figures in vision[8]. Thus these localisation patterns represent strong tendencies, rather than absolute rules.

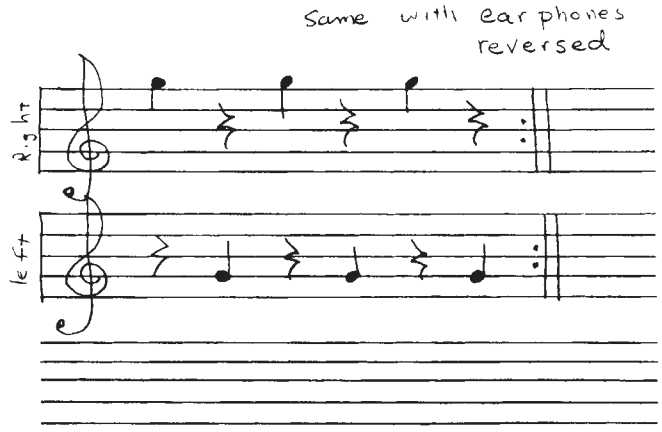

Fig. 2 Percept of the stimulus depicted by a subject with absolute pitch. The writing is that of the subject herself. Her statement: 'Same with earphones reversed' shows that the high tones were localised in the right ear and the low tones in the left, irrespective of the positioning of the earphones.

Table 2 Patterns of apparent localisation for the two pitches at the two ears in subjects who obtained the octave illusion

	RR	LL	Both
Right handers	25	5	1
Left handers	6	7	4

Each subject was twice given a 20-s presentation of the stimulus sequence, with earphones placed one way initially and then reversed. The order of earphone placement was strictly counterbalanced for both right and left-handed subjects. Figures show the number of right or left-handed subjects obtaining a given localisation pattern. RR, high tone localised in the right ear and low tone in the left on both stimulus presentations. LL, high tone localised in the left ear and low tone in the right on both stimulus presentations. Both, high tone localised in the right ear and low tone in the left on one stimulus presentation; and high tone localised in the left ear and low tone in the right on the other.

The question arises whether a given localisation pattern depends on absolute or relative pitch levels in the stimulus configuration. To determine this, I selected twelve right handers who had consistently localised the 800 Hz tone in the right ear, and the 400 Hz tone in the left, showing no tendency to reverse this pattern over several listening sessions. These subjects were presented with tape segments consisting of tones alternating between 400 Hz and 800 Hz, between 200 Hz and 400 Hz, and between 800 Hz and 1,600 Hz, the other parameters being identical. These sequences were presented for 20 s each in counterbalanced order, with earphone positions also counterbalanced. Except for one subject's report on the 200 Hz–400 Hz sequence, the higher of the two tones in each pattern was always localised in the right ear and the lower in the left. I conclude that these localisation patterns depend on the pitch relationships between the competing tones, and not on a pattern of ear preference at different pitch levels. Further, when I presented the two sequences channelled through spatially separated loudspeakers instead of earphones, the illusion was still obtained;

even though both sequences were now presented to both ears, with only localisation cues to distinguish them.

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Maternal behaviour and the effects of stress in tree shrews

THE tree-shrews (family Tupaiidae), small squirrel-like mammals occurring throughout South-east Asia, are currently classified[1] into two subfamilies: Ptilocercinae (nocturnal pen-tailed tree-shrews; genus *Ptilocercus*) and Tupaiinae (diurnal tree-shrews; genera *Tupaia, Anathana, Dendrogale, Lyongale, Urogale*). The overall systematic status of the family is still controversial. The tree-shrews are variously allocated to the Primates[2-3], Insectivora or to an entirely separate order of mammals[4-6]. Recently the unusual maternal behaviour of one tree-shrew species, *Tupaia belangeri*, together with other reproductive characteristics, has been interpreted as further evidence excluding tree-shrews from the Primates[1]. Key features of maternal behaviour in captivity are that the mother deposits her young in a separate nest subsequently visited for suckling only once every 48 h. If such behaviour and its functional correlates are typical of all tree-shrew species, arguments based on reproductive peculiarities could be more confidently integrated with considerations of methodology, general morphological evidence[7], detailed information on the central nervous system[8] and the results of biochemical investigations[9] to reach a balanced assessment of the phylogenetic relationships of tree-shrews.

A comparative study has now been carried out by one of us (F.D'S.) on the reproduction of three tree-shrew species representative of the adaptive range within the subfamily Tupaiinae (possibly excluding *Dendrogale*). The study included one of the largest, predominantly terrestrial species *Lyonogale tana* (average body weight 200 g), the intermediate, semiarboreal *Tupaia belangeri* (*cf. T. glis* average body weight 145 g), and the smallest, extensively arboreal species *Tupaia minor* (average body weight 44 g)[10]. Details of laboratory maintenance and observation are provided by Martin[1]. In the period May 1971 to February 1973, twenty litters were born (a total of forty-three infants: sixteen *T. minor*; sixteen *T. belangeri*; eleven *L. tana*). Thirty (70%) of the infants survived to sexual maturity (thirteen *T. minor*; ten *T. belangeri*; seven *L. tana*). Data have also been collected on three additional litters (six infants) of *T. belangeri* reared by their mothers in Martin's laboratory.

One of us (F.D'S.) has now demonstrated, under established conditions[1], that both *T. minor* and *L. tana* also typically exhibit 48-h suckling intervals whilst rearing their young in separate nest-boxes (Fig. 1). Accordingly, weight curves (Fig. 2) show a characteristic zig-zag pattern, since the milk continues to be digested without replenishment over each 48-h period. *L. tana* and *T. minor* infants, like *T. belangeri*, maintain a constant body temperature of about 36° C from birth onwards. With all three tree-shrew species, the amount of milk given to the litter by the mother at each suckling visit is considerable (*L. tana* average, 9.4 g; median, 9.4 g; *T. belangeri* average, 4.8 g; median, 5.0 g; *T. minor* average, 1.8 g; median, 2.0 g). Analysis of the milk of the three species shows that both the calorific density and the fat calorie content are extremely high (total solids: *L. tana*, 33.5%, *T. belangeri*, 40.4%, *T. minor*, 44.6%. In all three species: fat, >17%; protein, 6–11%; lactose, <2.5%.) It has been suggested[1] that this milk composition is necessary for constant maintenance of an elevated body temperature by the young. In addition, the solid content of the milk is inversely proportional to the adult and infant body size of the species concerned. The high protein content of the milk is consistent with the normal rapid growth rate of the young (Fig. 2). In all three species, as reported for *T. belangeri*[1], each suckling visit made by the mother is very short. The quantity of milk (q) given increases with the species' body size, but the duration (d) of the suckling visit decreases with increasing body size, means for 3 litters each: *L. tana* q=9.4 g (2.0–19 g) (1.0–11 m); d=3.9 min; for *T. belangeri*, q=5.0 g (1.0–10 g); d=5.8 min (2.5–9.5 m); for *T. minor*, q=2.0 g (1.0–6.5 g); d=6.6 min (2.5–10 m). The small litter sizes of the three species (usually one to three) are probably

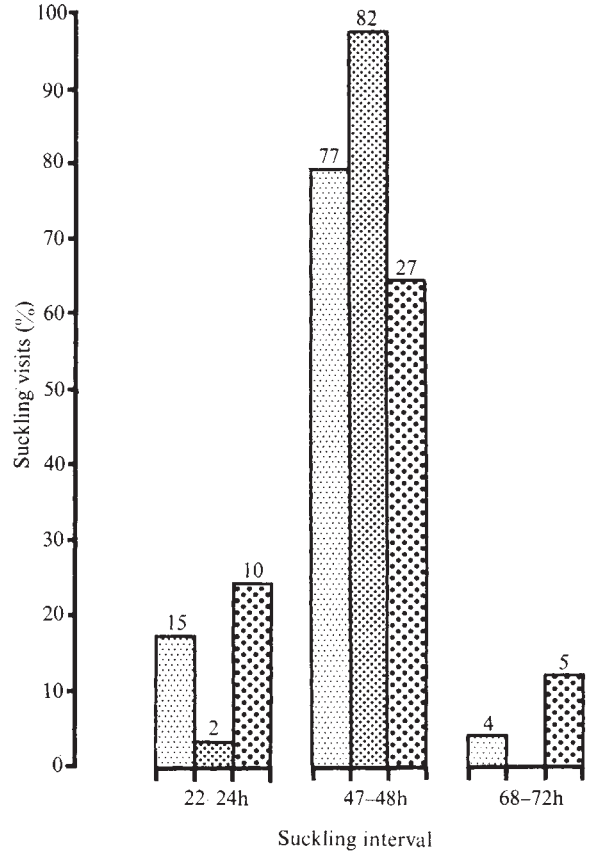

Fig. 1 Histogram of suckling intervals for the three tree-shrew species. Fine stippling: *T. minor*; Medium stippling: *T. belangeri*; Coarse stippling: *L. tana*. The three limited ranges shown on the abscissa cover all the observed suckling intervals. Numbers above the bars indicate numbers of observations.

constrained by the minimum infant body weight consistent with maintenance of a constant elevated body temperature (compare Fig. 2) and by the upper limit of milk production by mother tree-shrews. In fact, one unusual litter of four infants was successfully reared by a *T. belangeri* mother; but the amount of milk given to the litter as a whole was little different from that given to typical litters of two or three young (average total amount of milk given during nest phase to three litters of two is 153.6 g (range 134–172 g); total given to litter of four was 181.0 g).

Thus this peculiar pattern of maternal behaviour is, under appropriate conditions, typical of *T. minor*, *T. belangeri* and *L. tana*, with only minor variations according to body size. Circumstantial evidence indicates that *Urogale everetti*[11] behaves similarly and, although no evidence is yet available for *Dendrogale* it seems that this 'absentee system' of maternal care might be typical for the Tupaiinae. It remains to be seen whether *Ptilocercus* possesses the same characteristics. Certainly, it is difficult to reconcile the pattern found in the Tupaiinae with the suggestion that tree-shrews are derived from the ancestral primate stock, since all living primates are characterised by the advanced condition of the young at birth and by the great elaboration of mother–infant contacts[12].

Sorenson[13] has recently questioned the occurrence of this pattern of maternal behaviour in tree-shrews and has suggested that the 48-h rhythm may be peculiar to *T. belangeri*.

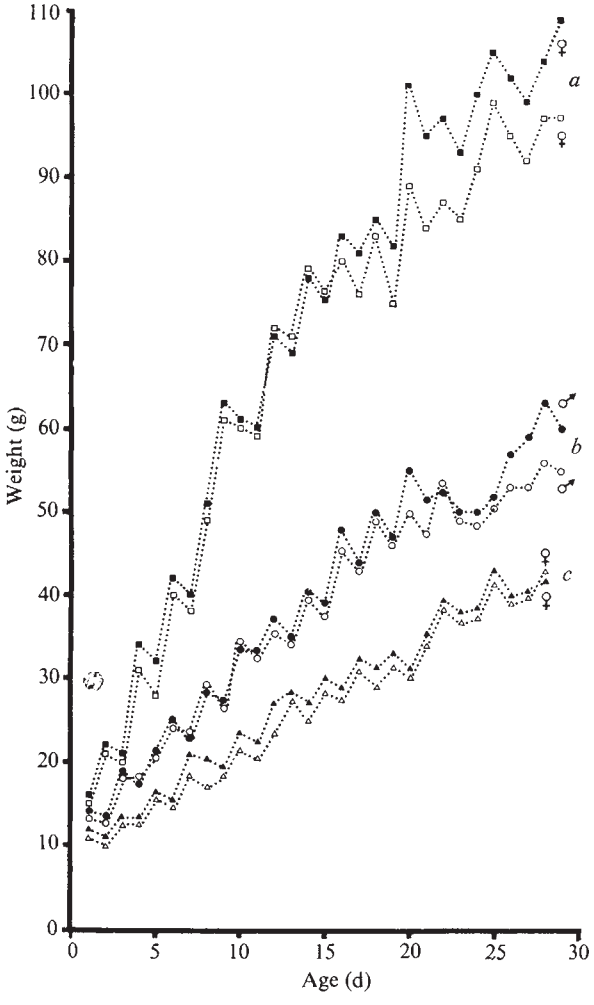

Fig. 2 Weight curves for single litters of two young per litter, for *L. tana* (*a*); *T. belangeri* (*b*); *T. minor* (*c*). The birth weights of the three species are relatively similar but weights diverge considerably by the end of the nest phase. The dip in *T. belangeri* (*b*) weights at 3 weeks coincided with the arrival of a new laboratory technician (an event known to disrupt tree-shrew maternal behaviour[1]).

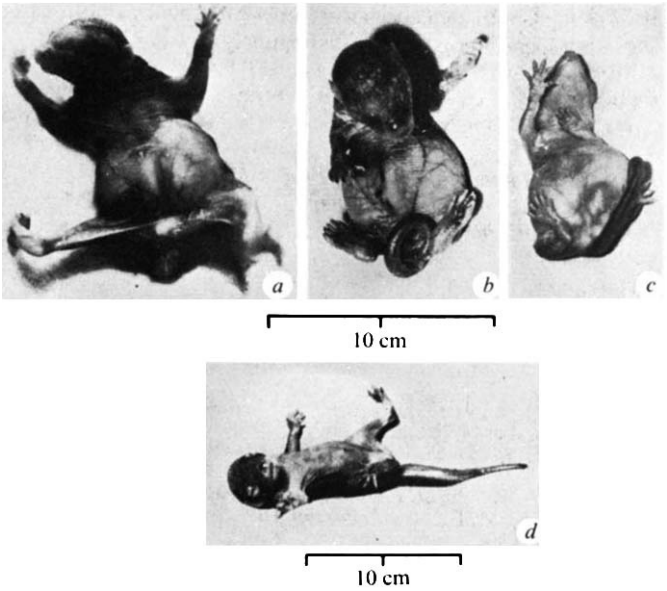

Fig. 3 Neonate *L. tana* (*a*); *T. belangeri* (*b*); *T. minor* (*c*), showing stomachs distended with milk ingested immediately after birth. At the end of each 48-h interval between suckling visits the stomach is deflated. (*d*: 10-d-old *T. belangeri* just before suckling). Note the altricial condition of the neonates.

Earlier careful studies, however, did not disclose such behaviour in this species and it is surprising that Sorenson did not observe similar behaviour with *Tupaia longipes*, a very close relative of *T. belangeri*. D'Souza's subsequent observations of the "absentee system" in both *T. minor* and *L. tana* (Fig. 3) conflict with Sorenson's account and a laboratory artefact may have been involved at some point. A clue is provided by von Holst's[14] observations that mild social or general environmental stress will distort maternal behaviour in *T. belangeri*, so that the suckling intervals become shorter but less milk is given over a 48-h period. (More severe stress leads to cannibalism of infants and eventually to sterility.) An example of such mild disruption was produced in Martin's laboratory by two fire-bell tests at a time when a female *T. belangeri* was rearing infants. After each fire alarm, the suckling rhythm accelerated, but the quantity of milk given over the 48 h was reduced by about 30% below expectation. The outcome of the two bell tests (Fig. 4) was that the development of the young was retarded (for example the eyes opened 3 d late and emergence from the nest was delayed by 4 d). Thus where the 48-h suckling rhythm has not been observed with tree-shrews breeding in captivity, the composition of cage groups and/or the general environmental conditions provided may inadvertently have led to modification of maternal behaviour. This interpretation is entirely supported by an examination of infant mortality rates. Martin achieved infant survival rates of about 80% with pairs of *T. belangeri*, kept under improved conditions[1], whereas D'Souza has attained survival rates of 70% with all three species. This compares with survival rates of 25% on average (mortality rates of 75%) with various colonies in which the 48-h rhythm was not apparent. An exception occurred with a colony maintained by Kuhn and Starck[15], where females were kept isolated after mating and where infant survival rates of 60% were achieved; but no data on maternal suckling behaviour were provided. In fact, Conaway and Sorenson[16] hand-reared many of their tree-shrew offspring because infant mortality was extremely high. In a study conducted by Sorenson and Conaway with *Tupaia montana*[17], the mortality rate of the offspring born in captivity was apparently 100%. If the survival rate can be taken as a criterion of the degree of immunity of maternal

behaviour from disruption in captivity, it seems likely that the 48-h suckling rhythm is the more 'natural' pattern, although no relevant field observations have yet been conducted.

As one further test of the 48-h suckling rhythm in *T. belangeri*, Martin removed infant tree-shrews five times from the nest for periods of about 36 h between suckling visits to see if this had any effect on maternal behaviour. The weight curves of the infants ranked in the upper range recorded for the species[1]. It is striking that total removal of the infants from the nest for long periods has virtually no effect on tree-shrew maternal behaviour,

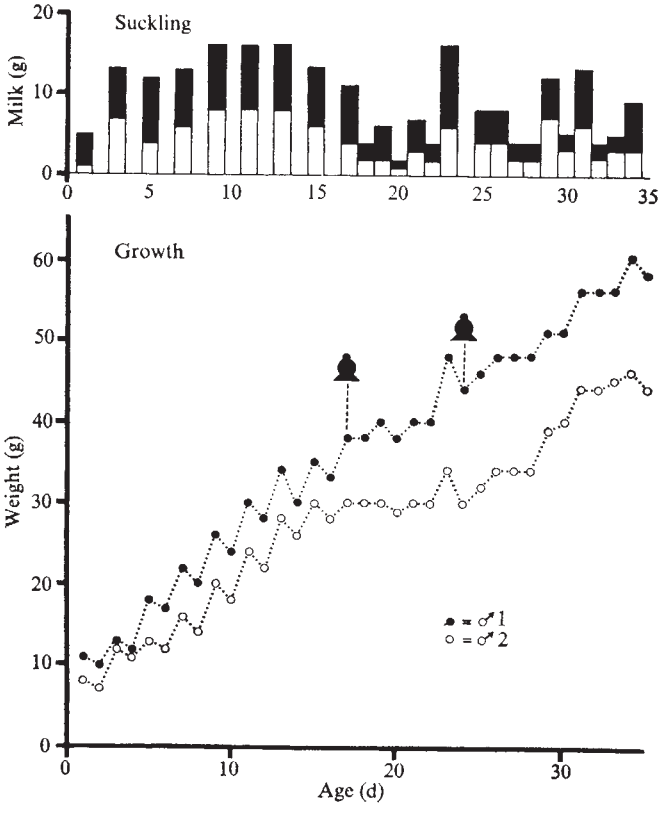

Fig. 4 Weight curves (lower) and histogram showing milk intake (upper) for a litter of *T. belangeri*. Two fire-bell tests were carried out on days 16 and 23, in both cases resulting in disruption of the 48-h suckling rhythm, reduction in milk intake per 24 h and depression of the weight curve. ■, ♂ 1; □, ♂2.

whereas two 30-s fire-bell tests can produce marked disruption. The results reported here should establish that the 48-h suckling rhythm is indeed typical of several members of the Tupaiinae when maintained under relatively stress-free conditions, whilst at the same time providing a cautionary tale for research workers concerned with the interpretation of behaviour in captive colonies of animals.

The analysis of tree-shrew milk was carried out by R. L. J. Lyster, National Institute for Research in Dairying, Herr K. Noack, Allgauer Alpenmilch Gesellschaft (after Martin[1]), and Dr D. L. Frape, Spillers Ltd. We thank Dr Gilbert Manley for a critical reading of the manuscript.

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Trace element content and body size in molluscs

WHEN trace element concentrations in shellfish are expressed on a weight-specific basis (as $\mu g\ g^{-1}$) highest values are often recorded in the smallest individuals[1]. In these cases, it is difficult to assess whether observed differences in element tissue concentrations between populations reflect real differences in environmental trace element constitution, or are merely due to variations in body size. This problem can be avoided by determining element concentrations over a range of body sizes and reference made to a specific size for comparative purposes.

Element concentrations have been measured in individual molluscs over a wide size range. Six estuarine species have been examined: limpets, *Patella vulgata* L. and *P. intermedia* Jeffreys; the cockle, *Cerastoderma edule* (L.); the mussel, *Mytilus edulis* L.; and clams, *Mercenaria mercenaria* L. and *Venerupis decussata* (L.). Limpets were collected from Portishead, on the Severn Estuary and the River Helford, Cornwall; cockles from Southend, Essex; *M. mercenaria* from Southampton Water; and *V. decussata* and *M. edulis* from Poole Harbour, Dorset.

Nitric acid digestion techniques were employed, trace element concentrations being determined by atomic absorption spectrophotometry in a final volume of 5–20 cm^3 1 M HCl (with additional dilution if necessary). Theoretical instrument sensitivities for the various elements in the minimum volume used of 5 ml are: Cd 0.05, Cu 0.1, Fe 0.5, Ni 0.5, Pb 0.5 and Zn 0.05 μg. Because such limits apply only to pure solutions, not subject to matrix effects found in digestates of animal tissues, minimum amounts considered were generally at least five to ten times greater than the above values. Concentrations obtained have been expressed as the content (μg per individual) and as the weight-specific concentration ($\mu g\ g^{-1}$). Both have been plotted against dry body weight (g). Regression lines were fitted by the method of least squares and all were significantly correlated at the 0.1% level.

The method of statistical analysis employed is analogous to that relating functions of metabolism to body weight[2,3]. Total trace element content per individual Y relates to body weight W as a power function expressed by:

$$Y = aW^b \quad (1)$$

which on logarithmic transformation yields a regression of the form:

$$\log_{10} Y = \log_{10} a + b \log_{10} W \quad (2)$$

The weight-specific relationship Y′ to body weight is essentially similar. Dividing (1) throughout by W yields:

$$Y' = Y/W = aW^b/W = aW^{b-1} \quad (3)$$

Logarithmic transformation produces a linear function:

$$\log_{10} Y' = \log_{10} a + (b-1) \log_{10} W \quad (4)$$

Clearly equations (2) and (4) have related regression coefficients and share a common intercept (Y and $Y' = \log_{10} a$ when $W = 1.0$, $\log_{10} W = 0$).

The precise relationship between trace element content (μg) and dry body weight (g) differs depending upon element and species. Three general relationships have been identified (Figs 1–4 and Table 1). First, element content is related to ≃ 0.75 power of body weight. This relationship was true for copper in all species examined. A variety of other elements exhibited the same relationship (Table 1), the actual values obtained for the regression coefficients of 32 lines (relating to the various elements in the six species examined) ranged from 0.67 to 0.85. Covariance analysis yielded no significant difference between pairs of coefficients. Pooling the sums of the squares and sums of the products of deviations from the regression[4] gave a common regression coefficient of $b = 0.77 \pm 0.04$ (weight-specific slope −0.23) (Figs 1 and 2).

Second, element content is directly related to body weight ($b \simeq 1.00$). Nickel in all bivalve species examined and also lead and zinc in *M. mercenaria*, iron and zinc in *V. decussata*

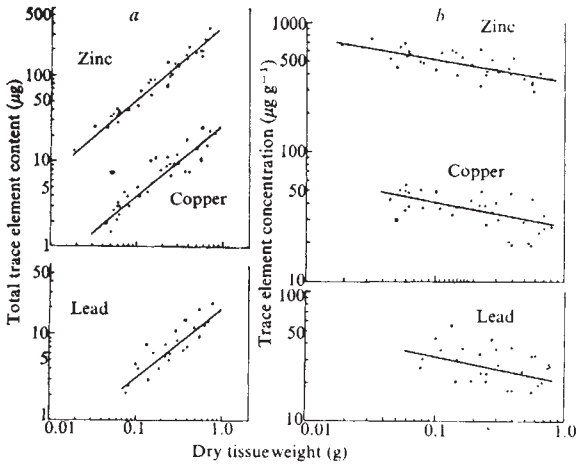

Fig. 1 Relationship of *a*, element content (μg) and, *b*, element concentration (μg g^{-1}) to body weight (g) for copper, lead and zinc in the limpet *Patella vulgata* collected on August 3, 1973 from Portishead, Severn Estuary.

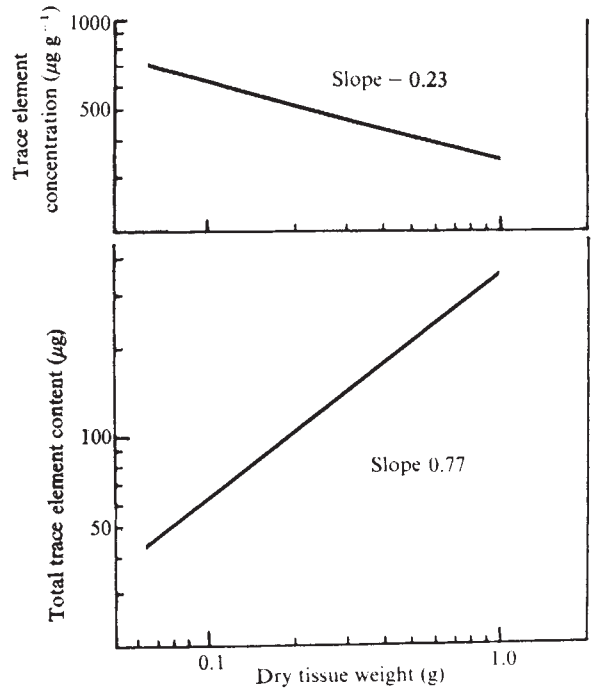

Fig. 2 Relationship of element content (and concentration) to body weight where element content related to 0.77 power of body weight. The data shown pertains to zinc in *P. vulgata* collected from Portishead, Severn Estuary.

and cadmium in *M. edulis* exhibited this relationship to body weight. The actual regression coefficients of 15 lines varied from 0.97 to 1.17; no pair of slopes being significantly different from one another. These lines yielded a common regression coefficient of 1.03 ± 0.02. In these cases tissue element concentrations are independent of size (Fig. 3).

Third, element content is related to square of body weight ($b \simeq 2.00$). This pattern was only observed for a single element, cadmium, in the limpet *P. vulgata* (Fig. 4). Therefore in this specific case, element tissue concentration is directly proportional to body weight.

Some earlier observations in the literature are consistent with the above relationships. A regression slope of 0.77 (weight-specific slope of −0.23) dictates that smaller individuals contain a higher concentration of metal than larger individuals. Such differences have been recognised for lead in *M. edulis*[1], zinc in *P. vulgata*[5] and copper and iron in *M. mercenaria*[6]. The converse situation, with higher concentrations of cadmium in large limpets compared with small individuals, has previously been reported for *P. vulgata* collected from the Severn Estuary[5,7]. In the molluscs studied, however, it was usual for element concentrations to decrease or remain constant with increasing body weight. Species from other phyla would be expected to exhibit similar relationships; certainly concentrations of

Table 1 Distribution of the three types of regression coefficients relating element content (μg) to dry body weight (g) within six species of mollusc

Regression coefficient	Cadmium	Copper	Iron	Nickel	Lead	Zinc
≃ 0.75	*C. edule* *M. mercenaria* *V. decussata*	*P. vulgata* *P. intermedia* *C. edule* *M. edulis* *M. mercenaria* *V. decussata*	*P. vulgata* *P. intermedia* *M. edulis* *M. mercenaria*		*P. vulgata* *P. intermedia* *C. edule* *M. edulis* *V. decussata*	*P. vulgata* *P. intermedia* *C. edule* *M. edulis*
≃ 1.00	*M. edulis*		*C. edule* *V. decussata*	*M. edulis* *C. edule* *V. decussata* *M. mercenaria*	*M. mercenaria*	*V. decussata* *M. mercenaria*
≃ 2.00	*P. vulgata*					

cadmium, copper, iron, manganese and zinc either decrease, or remain constant, in whole fish when expressed as a function of size, weight or length[8].

In molluscs, as in biota in general, absolute tissue element concentrations are related or determined by environmental concentrations. Within each species, however, different elements display different relationships to body weight (Table 1). Regression coefficients related to body weight by a power of 0.77 implies a connection with metabolism, as many metabolic processes display a similar relation to body weight. The common regression coefficient of 0.77 $\pm$ 0.04 is not significantly different from the coefficient of 0.75 $\pm$ 0.015 describing the relationship of respiration of poikilotherms to body weight[9]; a value generally accepted to relate many metabolic functions to body weight. Thus in cases where an element is related to 0.77 body weight some aspect of metabolism could well be influencing final trace element content.

Where the element content is directly related to body weight, as has been found for a variety of elements in several species, then a function of body weight such as the binding of specific compounds within the tissues may play some role in determining the total body element burden. The absolute amount of metal, however, is not dictated by the amount of such binding compounds within the tissues. At least certain metals such as cadmium and nickel in *Mytilus*, retain a specific slope relation of $\simeq$ 1.00 between total body element content and body weight, even though the absolute amount of the metals within the tissues differed by twelvefold for cadmium and twofold for nickel between two separate populations studied.

A relationship to the square of body weight as shown by cadmium in limpets can best be explained as being due to removal of this element from body circulation and accumulation within specific tissues, possibly as a result of some exceptional affinity. The slope value of 2.00 in the limpet population examined, may well be due to the unusually elevated environmental cadmium concentration in the Severn Estuary. Populations of limpets from other areas, where environmental cadmium concentrations are lower and more normal, exhibit slopes between 1.3 and 1.5 which may well indicate that in this specific case, the slope is variable and related to the environmental cadmium concentration.

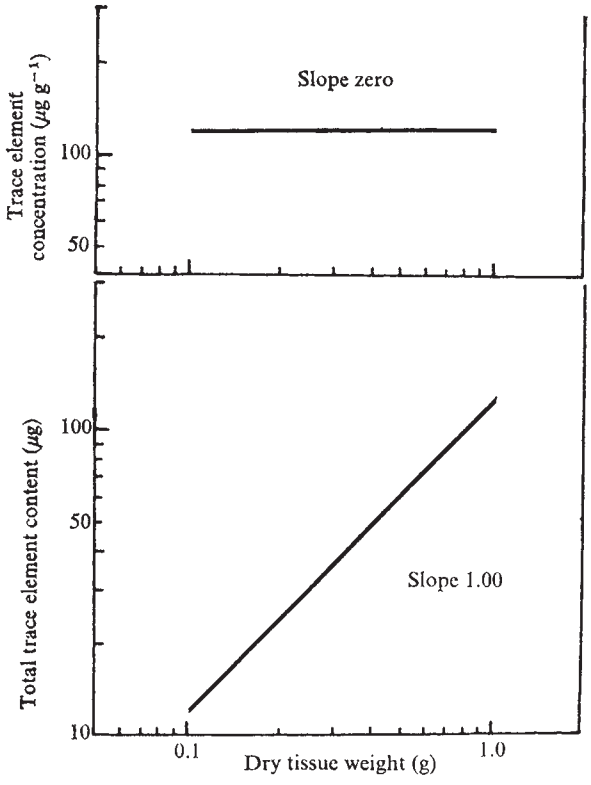

Fig. 3 Element content directly related to body size; weight-specific concentration independent of size. This example relates to nickel in the cockle *C. edule*.

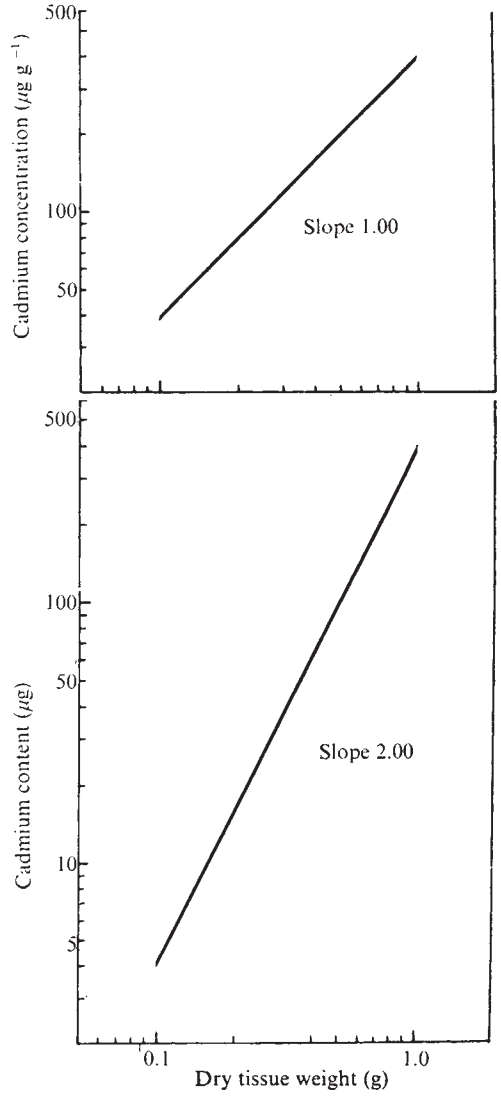

Fig. 4 Relationship of cadmium content (and concentration) to body weight in the limpet *P. vulgata* where cadmium tissue concentration (μg g^{-1}) is directly related to body weight. Limpets collected from Portishead.

Knowledge of the regression coefficients of lines relating trace element content (or the weight-specific concentration) to body weight is potentially a considerable aid in the monitoring of metals within shellfish. Evidence is being gathered which suggests that in most cases the regression coefficient relating element content to body weight remains constant irrespective of season or environmental element concentration. The only case so far identified where the slope differs between populations is that for cadmium in *P. vulgata* discussed above, though other exceptions may also be expected. If values of b are indeed constant for a particular element within a species, data pertaining to element content or concentration and mean tissue weight for a sample of similar sized individuals may be substituted, together with the appropriate slope for the element and species in question, into equations (2) or (4). The calculated intercept ($\log_{10}a$) converted to real values may then be used for comparison between populations irrespective of the size of the individuals sampled and analysed. The influence of seasonal variations and environmental element concentrations on the above relationships is receiving further investigation.

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LSD treatment of *Pieris brassicae* and consequences on the progeny

AT 20° C, under white light illumination and circadian rhythm, nymphal diapause of *Pieris brassicae* is dependent on a short 9-h photophase. Some psychodysleptics (mescaline sulphate and LSD), when injected during the photosentitive larval period, suppress diapause induction as if the larvae were subjected to a long 16-h photophase[1]. Abnormalities in the behaviour of the progeny of treated animals were observed. We studied them for three generations after the treatment.

Three couples of *P. brassicae*, progeny of a single original pair, were isolated. The progeny of each of these pairs were divided into two groups, one served as control (normal stock) and the other was treated with LSD. Control and treated animals of each progeny, respectively, were pooled at the end of each experiment after assessment of the fluctuation. Both stocks were subjected to a 9-h white light photophase treatment at 20° C (14,600 erg cm^{-2} s^{-1} with a Mazda TFR/40/BBL lamp).

LSD was diluted in Ringer solution to different concentrations. Animals were injected with 5 μl of the solution 24 h after the fourth moult.

As previously described[1], injection of 20 μg of LSD per animal results in about 80% of continuous development and 20% diapause induction. In the parental generation the LD_{10} was 35 μg per animal.

Among non-diapausing animals, different crosses were performed (Table 1). The normal partners were taken from the normal stock defined earlier.

The first striking observation was that all these F_1 larvae were highly resistant to LSD with a LD_{10} higher than 60 μg per animal. Second, 100% of the diapausing progeny of cross B, whether or not injected with 20 μg of LSD, died either at the end of the chrysalid stage or as malformed imagos (Table 1, B_1 and B_2, respectively).

Among diapausing animals, new crosses were performed (Table 1). Normal animals were taken from the progeny of the normal stock defined earlier. The sensitivity of the F_2 larvae to LSD in the three types of crosses seemed very high: the LD_{10} fluctuated between 5 and 10 μg per animal.

Different effects resulting from a treatment by LSD are apparent in the progeny of treated animals: an altered response to a 9-h photophase and the sensitivity to toxic effects of the drug. Two types of toxic effects have been detected. One is a short term effect measured through LD_{10}, the second is a long term effect apparent at the imaginal moult in diapausing animals.

The progeny of treated males and females (Table 1, B_1 and B_2) die at the imaginal moult although the egg laying ability of parents and hatching percentage, which were measured during all these experiments were found to be normal.

The early toxic effect of LSD follows a curious pattern with a LD_{10} of 35 μg for the parents, of more than 60 μg for the F_1 and 5–10 μg for the F_2 and the F_3.

The high resistance to LSD of the second generation may be explained by a detoxification mechanism, consequence of the parental treatment, enhanced by a further LSD treatment which could explain the slight improvement of viability in column B_2. Analysis of the effect of LSD on diapause induction must take the existence of such a resistance mechanism in account.

Table 1 Effect of LSD on diapause induction by a 9-h photophase

Parents	Treated	Not treated	Control (Ringer)
Total number	378	312	59
% diapausing	18	78	86

F_1	A treated ♂ × normal ♀		B treated ♂ × treated ♀		C normal ♂ × treated ♀		Control
	A_1	A_2	B_1	B_2	C_1	C_2	
Total number	77	111*	60	101*	91	128*	312
% diapausing	99	100*	25	97*	79	48*	78

F_2	A_1 ♂ × normal ♀	C_1 ♀ × normal ♂	C_2 ♀ × normal ♂
Total number	107	133	110
% diapausing	45	57	65

* When some animals were treated again, their total number and percentage of diapause are given (F_1 column 2).
Amount of LSD injected per animal was 20 µg. The control value in F_1 is taken from the parents.

The untreated progeny of treated males and females (B_1) show 25% of diapause induction only. This 'parental effect' is only observed if both parents were treated and completely disappears after a 20 μg LSD treatment (B_2). Such an induced resistance to LSD is asymmetric. If only one of the parents is treated and if this parent is the male, then treatment of the progeny has no effect (A_1/A_2). If this parent is the female, then a slight shift toward photophase resistance after LSD treatment can be observed (C_1/C_2).

We must then conclude that the resistance mechanism observed in the F_1 is very efficient when transmitted by males (A_1) and less efficient when transmitted by females (C_1). The LSD triggered enhancement of the resistance to the drug seems to be transmitted only by the males (A_2 and B_2) and is only apparent when a parental effect is present (B_1/B_2).

This parental effect can be observed in the F_2 generation even if only the grandfather or the grandmother was treated with LSD (results are statistically different from the normal 80% of diapause induction with less than 1% chance of error). We are apparently able to observe this transmission because the resistance mechanism, which in the F_1 masked the parental effect, is absent in the F_2.

The most probable target for LSD is the genetic material present in the germinal cells of the parental generation. Successive crosses with normal animals lead to a dilution of treated genetic material and a progressive shift toward a normal situation.

Damage to chromosome structure after LSD treatment has been described in different animals such as mice,

humans and amphibians[2-6]. Unfortunately such damage is difficult to detect in Lepidoptera.

If what we have found in an insect is also true in mammals then we should observe a high resistance to LSD in the progeny of treated parents and physiological effects detectable even several generations after treatment could be predicted as these are common features of the different crosses we have performed. This could be confirmed in mice.

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Regions of the freezing curve causing changes in structure and viability of ram sperm

WHEN ram spermatozoa are chilled and frozen, their acrosomes are damaged even in the presence of protective substances in the diluent. It has been suggested[1] that these structural changes contribute to the reduced fertility of frozen ram semen. Although the motility and structure of spermatozoa are affected to different extents by freezing it is not known if these changes occur simultaneously or are caused at different stages of the freezing process.

The present work was designed to identify the critical regions of the freezing curve by assessing the motility of and acrosomal damage to spermatozoa after cooling to various temperatures below zero. The experimental design was a 4^2 factorial array of temperature to which the sample was cooled (−5° C, −15° C, −45° C and −196° C) and level of egg yolk in the diluent (0%, 0.15%, 0.75% and 3.75% v/v). Samples of ram semen were diluted in a buffered glucose solution similar to that described previously[1] except that the glucose content was increased from 185 mM to 247 mM. The semen was diluted at 30° C and then cooled at a constant rate of 0.21° C min^{-1} to 5° C. An equal volume of diluent containing glycerol was added at 5° C and the diluted semen was held at this temperature, for not less than 5 h. The final dilution rate was 20-fold. Semen was frozen in plastic straws (0.25 ml, Cassou, l'Aigle) at a constant rate of 12° C fall min^{-1} to −55° C in a controlled freezing device. For lower temperatures the straws were transferred at −55° C to liquid nitrogen (−196° C). The order of the freezing treatments in successive replications of the experiment was determined according to a Latin square design. By this means, the main effect of freezing treatments was separated from the possible influence of differences in the equilibration time at 5° C.

The samples were thawed 10 min after reaching the final temperature. All samples were examined under the microscope within 5 min of thawing, and motility was estimated on a subjective scale of 0–4 (ref. 2) and the percentage of motile spermatozoa was estimated to the nearest 10%. Smears of frozen–thawed semen were fixed, stained and scored as described by Watson and Martin[1], who scored acrosomal damage on a 0–3 scale (0 for an undamaged acrosome and 3 indicating loss of the acrosome).

The results and statistical analyses are shown in Table 1. A significant decline in the motility and percentage of motile cells

Table 1 Mean survival and acrosome scores for ram spermatozoa cooled to different temperatures in diluents containing various levels of egg yolk

	Treatment	Motility (Scale, 0–4)	% Motile	Acrosome score (Scale, 0–3)
A	Temperature to which cooled (° C)			
	1 −5	2.44	45.94	1.11
	2 −15	1.86	30.94	1.51
	3 −45	1.63	27.34	1.23
	4 −196	1.80	30.47	1.12
B	Egg yolk level (%)			
	0	1.42	22.19	1.32
	0.15	1.92	32.50	1.30
	0.75	2.28	43.44	1.22
	3.75	2.09	36.56	1.13

Summary of analyses of variance

	Source of variation	Degrees of freedom	Variance ratios: Motility	% Motile*	Acrosome score
A	Cooling treatments				
	1 against 2, 3 and 4	1	37.93†	58.04†	8.75‡
	2 against 3 and 4	1	1.62	1.20	29.13†
	3 against 4	1	1.62	2.20	2.63
B	Egg yolk				
	Egg yolk against none	1	37.93†	54.77†	3.00
	Levels linear	1	1.62	2.37	5.50§
	quadratic	1	5.52§	15.86†	<0.01
	Equilibration	3	1.03	0.36	2.13
C	Ejaculates¶	7	4.14†	10.43†	3.13‡
	Pooled first-order interactions of treatments	51	0.49	0.80	1.19
	Residual (error) variance	60‖	*0.29*	*43.27*	*0.08*

* Data transformed to angles for analysis.
† $P < 0.001$; ‡ $P < 0.01$; § $P < 0.05$.
¶ 2 ejaculates were used within each replication row of the Latin square.
‖ 63-3 (for equilibration effect) degrees of freedom for 'error'.

after cooling and warming was seen in those samples cooled to −15° C or below, compared with those cooled only to −5° C ($P < 0.001$). Crystallisation of the external medium occurred at −12° C. Samples cooled to −5° C were therefore supercooled but not frozen; all other samples were frozen. This probably accounts for the reduction in survival of frozen samples since no difference in survival rate was detected between samples cooled to −15° C, −45° C and −196° C. Spermatozoa frozen to −15° C or below showed a greater mean acrosomal deterioration than those supercooled to −5° C ($P < 0.01$), but freezing to −45° C or −196° C was less detrimental than freezing to −15° C ($P < 0.001$). The greatest changes in the acrosomes were seen in those spermatozoa frozen to −15° C. Damage to acrosomes did not result from crystallisation of the suspending medium, however, since the degree of damage was similar in the samples cooled to −5° C and −196° C. Dehydration of the cells with consequent salt concentration, and intracellular ice formation and recrystallisation could all be factors contributing to the damage since these events could occur during the 10 min pause before rewarming. They are less likely to occur at lower temperatures because of the lower energy state of the sample.

The presence of egg yolk in the diluent improved the survival of spermatozoa after cooling and warming ($P < 0.001$). Survival with 0.75% egg yolk was significantly greater than with either the higher or the lower level of egg yolk ($P < 0.05$—motility score; $P < 0.001$—percent motile score). Acrosomal damage was decreased by increasing levels of egg yolk ($P < 0.05$). More egg yolk was thus required to protect the acrosomes, than to preserve motility. It may be that the stresses induced by chilling and freezing in the large surface area of the membranes surrounding the head of the spermatozoa are greater than those

in the region of the midpiece. If a membrane-coating hypothesis is advanced for the protective action of egg yolk, the more labile membrane structures of the acrosome may require a greater concentration of egg yolk in order to stabilise them than do those involved with the movement of the spermatozoon. A similar pattern of response to the egg yolk levels was shown by the samples cooled to $-5°$ C, to that seen in those cooled below the freezing point, since the 'cooling temperature × egg yolk level' interaction was non-significant for all scores. This supports the view that egg yolk plays a protective role during and after freezing similar to that before freezing.

Bank and Mazur[3] discussed the fact that there is no simple correlation between the survival and the degree and type of structural alteration of cells during freezing. For ram spermatozoa under the conditions in this experiment, motility was mainly affected by the phase change occurring near to $-12°$ C, but the critical region, in which structural changes occurred, extended from this temperature to about -45 °C. It may well be that the freezing curve which allows maximum survival with minimum structural alteration has different cooling rates for different regions of the curve.

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Proteoid roots are microbially induced

ALTHOUGH some workers[1] have examined environmental factors affecting the formation of dense clusters of rootlets ('proteoid roots') in many taxa of the Proteaceae, the causal agents have not been elucidated. The basic phenomenon is an intense, localised production of short lateral roots occurring particularly in nutritionally poor conditions. Purnell[2] and Lamont[3] were unable to detect bacteria, actinomycetes or fungi in newly formed proteoid roots although microorganisms were sometimes found in older roots. Here we demonstrate that proteoid roots are caused by non-infecting rhizosphere microorganisms.

Nine hundred gram lots of two soils poor in nutrients (a podzolised soil, Mount Burr sand, South Australia[4] with 3 p.p.m. sodium bicarbonate extractable P; and a lateritic podzolic soil from Dwellingup, Western Australia[5] with less than 1 p.p.m. extractable P) were wetted to 70% field capacity with distilled water and placed in lidded polythene pots. Watering tubes of polythene tubing (1 cm diameter) with the lower end buried 2 cm into the soil were sealed into the lid, and the open end was covered with a metal cap. The assemblies were sterilised by γ irradiation (5 Mrad, Minster Carpets, Dandenong, Victoria). Seeds of *Banksia grandis* Willd. were surface sterilised and germinated on a nutrient agar[6]. Two sterile seedlings were planted in each pot by inserting the radicles through slits cut aseptically in the lid of the pot, and then sealing with petroleum jelly. Microbial inoculum was prepared by shaking 10 g of fresh proteoid roots (with attached rhizosphere soil) of *B. grandis* from a nutrient-poor soil in 200 ml sterile distilled water and 1 ml of this suspension was introduced through the watering tube to each 'inoculated' pot and 1 ml of sterile distilled water to uninoculated controls. Watering throughout the study was with sterile distilled water introduced by the capped water tubes. The plants were randomly distributed in a controlled environment growth cabinet and harvested after 4 months' growth at 20° C, with a 12 h day with light from Osram mercury vapour lamps (MBFR/u, 400 W, General Electric Company, London), providing 20,400 lumen m^{-2}.

In Mount Burr sand, none of the plants in the 12 sterile control pots produced proteoid roots while the plants in all 12 of the inoculated pots did; proteoid roots were particularly marked around the point of inoculation. In the lateritic podzolic soil none of the plants in the nine replicate control pots which remained sterile formed proteoid roots while all plants in the 12 inoculated pots did. The plants in the three microbially contaminated pots produced proteoid roots. None of the proteoid roots sectioned from inoculated pots was infected and we conclude that growth of microorganisms in the rhizosphere triggered formation of proteoid roots by the plant. Some microorganisms are known to produce growth substances affecting root morphology after infection by them[7,8] but this seems to be the first report of a major change in root morphology by non-infecting rhizosphere microorganisms.

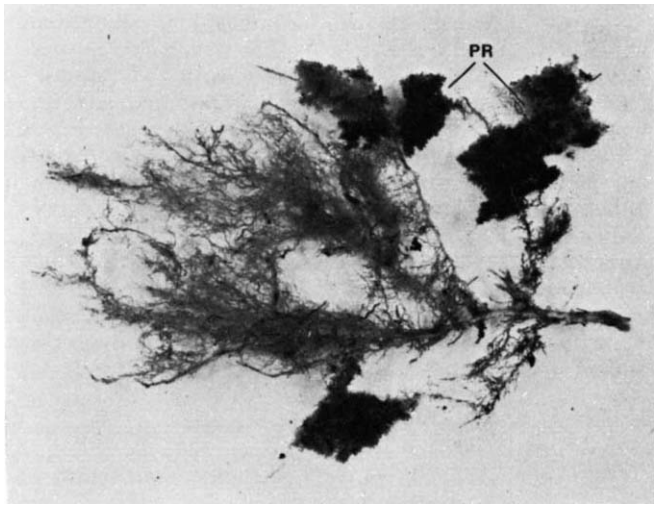

Fig. 1 Clusters of proteoid roots (PR) *Banksia grandis* (×0.355).

Direct microscopic observation of proteoid roots from inoculated pots following staining[9] showed the rhizosphere to be dominated by bacteria. Counts of total bacteria, total aerobic viable bacteria, pseudomonads[10], Gram-negative bacteria[11], and *Bacillus spp.* (after heating dilutions for 10 min at 80° C) showed the rhizoplane numbers and frequency of these groups to be similar per mg of root with proteoid roots and corresponding non-proteoid roots on the same plant. Thus, the above counts did not give any suggestion of the identity of the causal organisms. Pure culture studies are proceeding. The production of proteoid roots in three pots with contamination infers that either a very common airborne organism or a wide range of organisms could induce proteoid root formation. If specific organisms are involved, however, it is not impossible that a few of them survived the γ-irradiation treatment.

Dry weight of shoots of plants with proteoid root and without proteoid roots respectively were 1.22 g and 0.67 g in Mount Burr sand (difference significant at $P<0.01$) and 1.40 g and 0.79 g in the lateritic podzol (difference significant at $P<0.01$). In each soil the weights of the roots of plants with proteoid roots and plants without proteoid

roots were almost identical. Proteoid roots accounted for approximately half the root weight in Mount Burr sand and approximately 40% of the root weight in the lateritic podzolic soil. Jeffrey[12] demonstrated the importance of dense surface mats of proteoid roots trapping sudden phosphate accessions and also attributed an increased uptake of phosphate from solution culture to their greater absorbing surface. Figure 1 indicates the intense exploration of soil afforded by proteoid roots, and this would be expected to enhance uptake of poorly mobile ions already in the soil[13] as well as trapping added nutrients. Baylis[14] and Bowen[15] have commented on the importance of mycorrhizal associations in compensating low root density in uptake of poorly mobile ions from soil.

Uptake of ^{32}P from 5×10^{-6} M KH_2PO_4 in 5×10^{-4} M $CaSO_4$ over 15 min at 20° C showed uptake of phosphate by proteoid roots to be 1.82 ± 0.18 (s.e.) $\times 10^{-13}$ mol cm^{-2} s^{-1} compared with $0.48 \pm 0.07\times10^{-13}$ mol cm^{-2} s^{-1} for similar young unsuberised non-proteoid roots (differences significant at $P<0.01$). Assuming the microbial component of uptake to be similar (as indicated by the similar microbial counts and composition above) the enhanced phosphate absorbtion by proteoid roots is due not only to their greater surface area but also to increased absorbing ability. Stimulation of phosphate uptake and translocation by noninfecting rhizosphere microorganisms has been suggested for other plant groups[16,17].

We see the results as being significant in demonstrating yet another system which plants have evolved for enhancing their nutrition in low nutrient soils, not by themselves but in association with soil microorganisms. Other, better known, examples are legume and nonlegume nitrogen fixation[18] and various types of mycorrhizas[19] increasing ion uptake. Lamont[1] demonstrated experimentally that low or unbalanced N and P status of the growth substrate enhances proteoid root formation in nonsterile conditions. Our demonstration that rhizosphere microorganisms induce proteoid root formation suggests a need to examine the extent to which observed differences between soils in proteoid root formation are due to soil microbiological differences rather than soil chemical and physical differences.

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Oxygen microenvironment and respiratory oscillations in cultured mammalian cells

OXYGEN transport in cultures of attached mammalian cells depends on the kinetics of gas diffusion through the overlaying medium. Working with liver parenchymal cells, Stevens[1] calculated that the oxygen requirements and energy demands of a monolayer culture could not be met by atmospheric pO_2 if the fluid overlay was deeper than 0.34 mm. McLimans[2] theorised that under the standard medium overlay of 1.5–5.0 mm, oxygen depletion by a respiring monolayer of liver parenchymal cells would exhaust the atmospheric oxygen supply at the plane of the attached cell sheet within 35 min. His model suggests that adaptive growth of cultured cells occurs only when respiration does not exceed the delivery of oxygen to the monolayer.

To test these theories and the validity of arguments linking oxygen availability with respiration, energy metabolism and the regulation of cell growth, we constructed microoxygen cathodes[3,4] and investigated microenvironmental pO_2 in the medium overlaying attached cultures of WRL-10A cells. This subline of the L-cell mouse fibroblast, isolated in our laboratory[5], was used because we already had information about changes in medium pO_2 and respiratory rates during regulated states of growth and differentiation of these cells in suspension cultures[6,7]. Cells were grown attached to optically flat 50 mm Petri dishes with 6 ml of Eagle's minimum essential medium (MEM) supplemented with 20% horse serum, antibiotics and glutamine. Dishes were placed on the stage of an inverted microscope inside an environmentally controlled chamber, and maintained at 35° C in a humidified atmosphere of 5% CO_2 and air. A Pt/Ir oxygen microcathode with a tip 1–3 μm in diameter and a corresponding resolution 6–20 μm in diameter, was secured in a micromanipulator clamp, and positioned in the centre of the objective field so that descent of the cathode could be observed. An Ag/AgCl reference anode placed in a saline well adjacent to the dish was joined to the medium by an agar bridge. Starting at the fluid–air interface, the cathode was

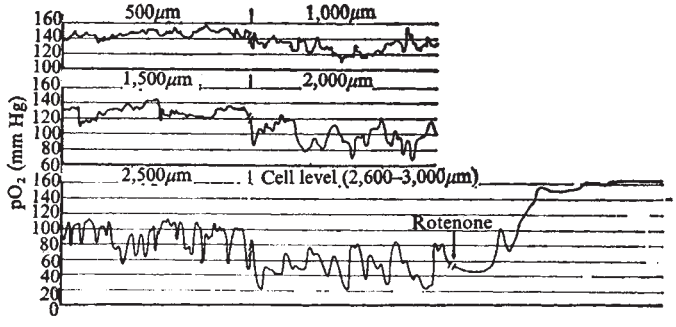

Fig. 1 Oscillations of microenvironmental pO_2 in the medium overlaying attached cultures of 10^7 cells per 50 mm Petri plate were measured amperometrically with calibrated Pt/Ir microoxygen cathodes polarised between 0.6 and 0.8 V. The oscillations were traced on a strip chart recorder connected to an electrometer which was attached in series to a cathode and a Ag/AgCl reference anode. The current output of these sensitive cathodes ranged between 0.5×10^{-11} and 0.5×10^{-12} A per mm Hg. Distances measured in micra represent depths from the surface of the medium towards the attached cell sheet. Oscillations at each depth are presented in comparable 30-min segments.

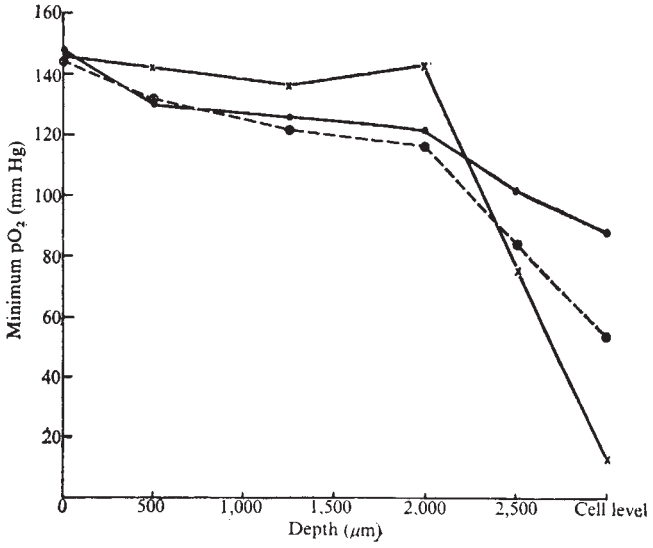

Fig. 2 Density-related oxygen gradients in the medium overlaying populations of attached WRL-10A cells. Experimental cultures contained 5×10^6 (●——●), 10×10^6 (○ - - - - ○), and 16×10^6 (×——×) cells per plate. Minimum pO_2 values represent means of the lowest pO_2 readings obtained from all oscillations recorded at each indicated depth.

lowered through the medium towards the attached cell layer and pO_2 changes were measured polarographically at selected depths. Pilot experiments detected the existence of oscillating concentration gradients of oxygen above the attached cell sheet. Because this oscillatory phenomenon had neither been predicted nor described in any literature on mammalian cell cultures we have tried to describe these dynamic oxygen fluctuations in detail, to determine their origins, to establish their relationship to cell density and to derive from them clues about oxygen availability as it relates to mechanisms controlling respiratory activity in attached populations of exponentially proliferating cells.

Using populations of 10^7 cells per plate, continuous recordings were made of the pO_2 oscillations at specific depths in the overlaying medium. A composite of the collected data (Fig. 1) shows that oscillation amplitude increased with depth. At 500 μm the oscillations were very shallow, at 1,000 μm and 1,500 μm they were slightly larger, but still relatively small while at 2,000 μm and 2,500 μm oscillations were mixed large and small. Continuous undampened oscillations with major and minor peaks occurred at cell level and their mean peak frequency ranged from 0.34 – 0.56 per min in different experiments. At each increasing depth, oscillations occurred within a characteristic range of progressively decreasing minimum and maximum pO_2. For example, at 500 μm they occurred between 140 and 160 mm Hg, while at the cell level they occurred between 20 and 80 mm Hg. Correspondingly, conditions of minimum O_2 availability at the various depths are described by a mean minimum pO_2 gradient, that is an oxygen gradient based on the average of the lowest pO_2 readings obtained from all the oscillations recorded at each particular depth. After recording the pO_2 oscillations at the cell level for 30 min (Fig. 1), 50 μl of a rotenone solution, 10^{-8} M in ethyl alcohol, were added to determine whether the oscillations were related to cellular respiratory activity. The effect of this respiratory inhibitor[8] was an immediate cessation of oscillations, followed by 9 min of maintained low pO_2, a last oscillatory rise and fall of local oxygen tension and a subsequent steady increase in pO_2. This brought the microenvironmental oxygen tension back into sustained equilibrium with atmospheric oxygen. Having thus demonstrated that the oscillations are an expression of cellular respiratory activity with oxygen depletion due to cell respiration and oxygen replacement due to diffusion, we investigated the effect of cell density on mean minimum pO_2 gradients.

Replicate cultures seeded with 7.0×10^5 cells per Petri plate were maintained by daily medium renewal and analysed polarographically at cell densities of approximately 5, 10 and 16×10^6 cells per plate. Comparisons of pO_2 gradients (Fig. 2) showed that from the air–fluid interface down to 2,000 μm, all three cell densities had media oxygen tensions that were relatively high and nearly in equilibrium with atmospheric pO_2. From 2,000 μm down towards the cell level, however, the oxygen tension decreased sharply in a directly inverse relationship to increasing density. Thus, the negative slope of the 5×10^6 cell population is the least steep and the slope of the 16×10^6 cell population is steepest. Likewise the mean minimum pO_2 at the cell level also decreased in direct proportion to increasing cell density, with the value of the 5×10^6 cell population reaching approximately 100 mm Hg, the 10×10^6 cell population reaching about 60 mm Hg, and the 16×10^6 cell population reaching about 19 mm Hg. Thus in attached cultures oxygen availability and microenvironmental O_2 gradients are directly related to the density and combined respiratory activity of the entire cell population.

When the amplitudes of the pO_2 oscillations were measured and their mean values at increasing depths compared (Fig. 3), the following correlations appeared. In cultures of 5 and 10 million cells the mean oscillation amplitude increased with depth down to 2,500 μm. At each depth the mean oscillation amplitude for the 10^7 cell population was significantly greater than the corresponding amplitude of the 5×10^6 cell population. Amplitude therefore increased as a function of both depth and increasing density. At densities of 16×10^6 cells and greater, the combined respiratory activity of the larger population intermittently reduced the microenvironmental oxygen supply at the cell level to extremely low levels, at which the respiratory activity seemed to be affected by oxygen limitation. Oscillatory behaviour in these high density populations is under investigation.

Our results indicate that the respiratory activity of WRL-10A cells under the condition of these experiments is periodic rather than constant. Furthermore, the density-dependent nature of

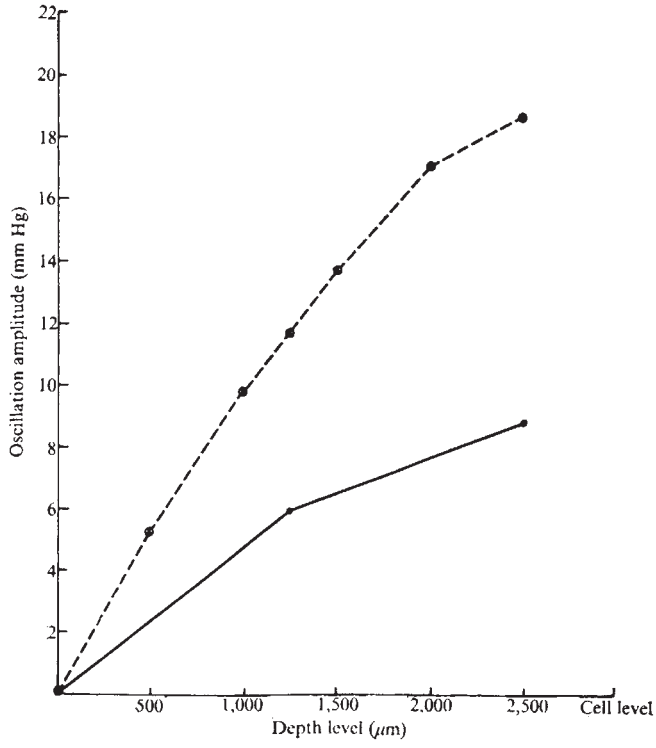

Fig. 3 Amplitudes of oscillations in exponentially growing cultures with 5×10^6 (●——●), and 10^7 (○ - - - ○) cells per plate. The values shown represent the means of all oscillations, large and small, recorded at each indicated depth.

the oscillations and of the media pO_2 gradients suggests that the entire cell population, or at least very large portions of it, respires in synchrony. This is evident because individual cells respiring periodically but out of phase, would tend to dampen or cancel out the oscillations. In the cellular microenvironment, the succession of predominantly undamped oscillations with minimum pO_2 values above 15 mm Hg reflects both the continuous cycles of partial depletion and renewal of microenvironmental oxygen and a control of respiratory activity that cannot be ascribed to mere periodic fluctuations of oxygen availability. We suggest therefore that cellular respiration in these cultures responds to environmental feedback[9] that is subject to modification by local exhaustion and diffusion of essential nutrients and metabolites and can alternately and synchronously turn the respiratory system on and off in a large number of cells. Such an oscillating system might consist of an energy balance between AMP, ADP and ATP or a fluctuation in some oxidisable substrate pool such as NADH, or a matrix of these and other interdependent variables such as pO_2, *p*H, pCO_2, glucose, Na^+ and K^+ all working in concert to control respiration and growth metabolism[10,11].

Stoker[12] demonstrated that populations of attached 3T3 cells, having reached confluency and stopped growing, can be induced into highly localised growth by gentle local agitation of the supporting medium. His inference is that the microenvironmental diffusion barrier proposed by Rubin and Rein[13] was reduced by the local disturbance thus changing the concentrations of essential growth factors or inhibitors and stimulating localised mitotic activity. Our data have provided direct demonstration of localised dynamic changes in the constitution of the growth media of attached cultures. The proposition that such changes may play an important role in growth regulation will be tested by extending these investigations to 'contact inhibited' cells.

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Developmentally regulated cell surface alterations in *Dictyostelium discoideum*

THE life cycle of the cellular slime mould, *Dictyostelium discoideum*, can be divided into two mutually exclusive phases[1]. The vegetative state, in which the cells divide by fission every few hours, is characterised by the absence of intracellular communication; whereas the developmental social state, which is initiated by the exhaustion of nutrients, exhibits many forms of such interactions. These include the ability to respond to and relay chemotactic signals[2], and to form stable species-specific cell to cell contacts[3-5]. The ability to form stable cell contacts can develop in the absence of a chemotactic gradient, as demonstrated by the immediate formation of normal aggregates upon plating of cells which have been maintained in suspension in a nutrient-free salt solution for 8–9 h (ref. 1). New antigens appear and function on the cell surface during development[3,4]. Gerisch and his coworkers have shown that univalent antibodies directed against the surface of 12 h cell aggregates interfere specifically with the formation of the stable cell to cell contacts[4-6]. Furthermore, it has been demonstrated that a developmentally controlled, heat-stable component, which is present in membrane preparations from 12 h aggregates, interferes specifically with the morphological and biochemical development of the organism (J.E.S., and J. Tuchman, unpublished, and J. Tuchman *et al.*, unpublished).

Most studies on cellular components which reside on the outer cell surface of the plasma membrane are complicated by the fact that the cell surface membrane must be disrupted in order to be purified and characterised. For some molecules on the outer cell surface this complication can be avoided by selectively labelling them before disruption of the cell[7,8].

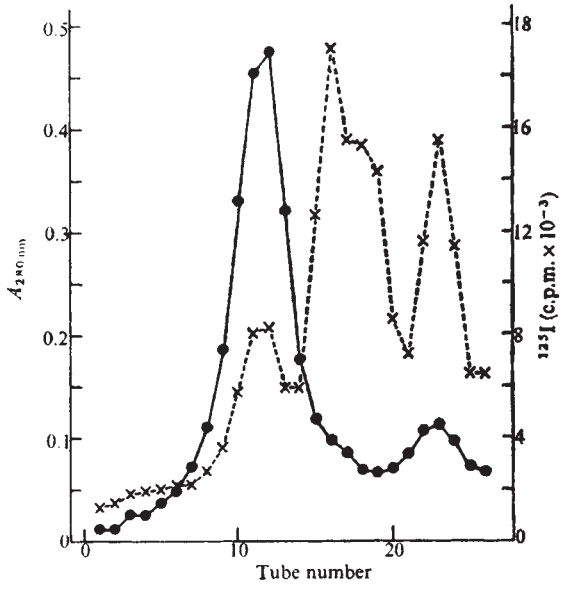

Fig. 1 Sucrose gradient profile of subcellular fractions of ^{125}I-labelled *D. discoideum* cells have formed firm aggregates 12 h after nutrient deprivation and plating. Cells were suspended at 10^8 cells ml^{-1} and carrier-free $Na^{125}I$ usually to a concentration of 400 μCi ml^{-1}. The reaction was initiated by the addition of lactoperoxidase (E.C. 1. 11. 1. 7) (Calbiochem) and glucose oxidase (E.C. 1.1.3.4) (Worthington Biochemicals) to final concentrations of 20 μg ml^{-1} and 0.1 units ml^{-1}, respectively. The reaction was allowed to continue for 10 min at room temperature with occasional swirling. The reaction was stopped by addition of phosphate buffered iodide, PBI (phosphate buffer saline with the NaCl replaced by NaI), and the cells were pelleted through a cushion of PBI + 10% glycerol. The resulting pellet was resuspended in 1 ml of PBS and frozen at 70° C. After thawing of the cells (which resulted in cell lysis), membranes were prepared by layering the suspension over a linear gradient of 0.5–2.0 M sucrose, 0.02 M Tris, *p*H 8.0, 0.005 M $MgCl_2$. The gradients were centrifuged at 23,000 r.p.m. for 18 h at 4° C in a MSE SW 6 × 14 rotor. ●, ^{125}I (c.p.m.); ×, A_{280nm}. The membrane containing turbid band was diluted with at least five volumes of 0.02 M Tris, *p*H 8.0, 0.005 M $MgCl_2$, and centrifuged at 30,000 r.p.m. for 30 min at 4° C in an MSE SW 6 × 14 rotor.

We used the $Na^{125}I$-lactoperoxidase–glucose oxidase coupled system[9] to monitor some of the developmentally controlled changes on the cell surface of *D. discoideum*. This method selectively labels proteins which have a tyrosine exposed to the extracellular aqueous environment. We observed that the plasma membrane of *D. discoideum* in its vegetative growth phase contains two major proteins (molecular weights about 135,000 and 55,000) which are on the external surface and accessible to labelling by the ^{125}I-lactoperoxidase system. After the cells have progressed through their developmental phase to the point of forming firm aggregates a new major protein (molecular weight about 130,000) becomes accessible to labelling. Other less prominent labelled proteins show quantitative changes in their availability to this reagent. *D. discoideum*, strain Ax-2, an axenic haploid strain[10], was grown to mid-log phase $3–5\times10^6$ cells ml^{-1}) in HL-5 medium[11]. The cells were collected and washed free of nutrients in cold pad diluting fluid (PDF)[12], and 5×10^7 cells were deposited on black Millipore filters (4.25 cm). After proceeding through their normal developmental phase for varying periods of time, the cells were harvested in cold PDF, washed once with phosphate buffered saline (PBS), and then with PBS+5 mM glucose. The cell surface was then labelled with ^{125}I (carrier-free $Na^{125}I$, Radiochemical Centre, Amersham, Bucks) using the $Na^{125}I$-lactoperoxidase–glucose oxidase coupled system as described in Fig. 1.

The labelled cells were lysed and fractionated on a linear sucrose gradient, as shown in Fig. 1. The lowest band in the gradient contains practically all of the TCA precipitable ^{125}I counts. This turbid band, when viewed under a phase contrast microscope, or by electron microscopy, is seen to consist of large membranous fragments, as well as smaller vesicular components[13]. Whereas longer centrifugation results in further sedimentation of the other bands, the position of the turbid ^{125}I-containing band is not affected, and it is, therefore, assumed to be at its isopynic density (about 1.16–1.17 g ml^{-1}) (J.E.S. and J. Tuchman, unpublished).

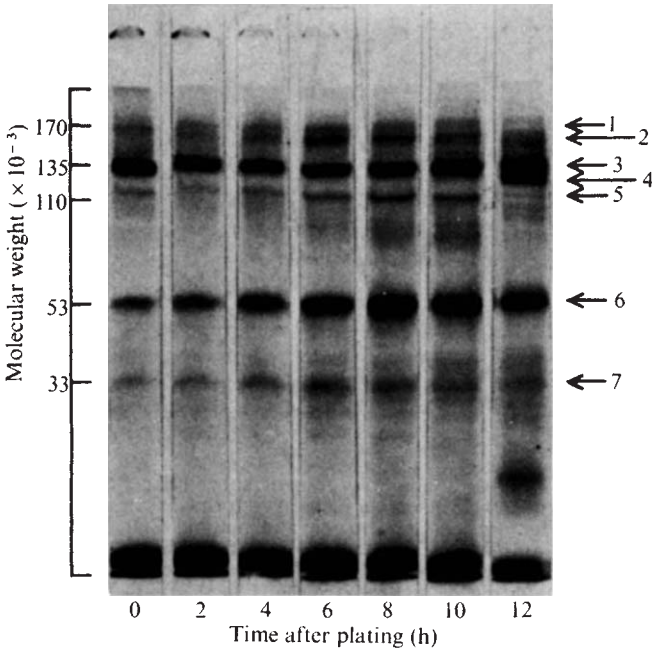

Fig. 2 Autoradiogram of 7.5% sodium dodecyl sulphate-polyacrylamide slab gel electrophoretogram of ^{125}I-labelled membranes of *D. discoideum* at various stages of normal differentiation. Membranes were dissolved in sample buffer containing 1% SDS 0.01 M dithiothreitol, 10% glycerol, and 0.05 M Tris, *p*H 6.8, by boiling for at least 2 min. Electrophoresis was done in slabs where 12 samples could be run in parallel on the same slab[16]. The buffer system used was that of Laemmli[17]. Electrophoresis was done at 50 V for about 1 h, until the bromophenol blue marker entered the separation gel, and then at 100 V (about 10 V cm^{-1}) until the marker reached the bottom. The slabs, after they were stained with Coomassie blue if required, were dried down on to paper, and autoradiographs made on Kodirex X-ray film.

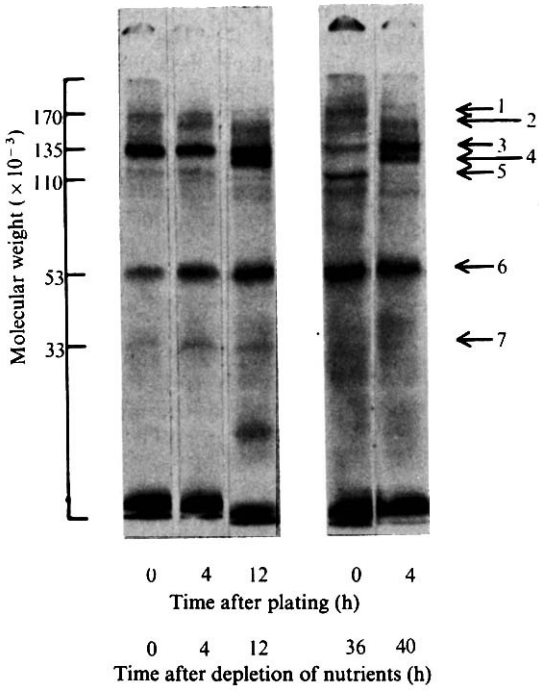

Fig. 3 Autoradiogram of 7.5% SDS-polyacrylamide slab gel electrophoretogram of ^{125}I-labelled membranes of *D. discoideum* at various stages of normal differentiation or at various stages of development of 'aggregation competence' in suspension culture. Samples for suspension culture were collected as described and resuspended at 10^7 cell ml^{-1} in PDF. The cells were maintained for various periods of time in shaking culture. In the above experiment the cells were maintained in liquid suspension culture for 36 h at which time one aliquot of 10^8 cells was labelled with ^{125}I. An identical aliquot was plated on black Millipore fitters and allowed to form firm aggregates (3–4 h after plating), before labelling.

In the absence of lactoperoxidase and glucose oxidase, the labelling is only 0.6% of the control level, suggesting that the reaction is totally dependent on the exogeneously added enzymes, which presumably do not enter the cell. Removal of 94% of the TCA precipitable counts by mild trypsinisation of whole cells (leaving the cells intact) also supports the conclusion that the label is only attached to proteins on the cell surface. If cells which have been mildly trypsinised after labelling are fractionated by sucrose gradient centrifugations essentially all of the remaining TCA precipitable counts are found with the soluble proteins. Furthermore, if the cells are lysed (freeze–thaw) before the labelling procedure, the ^{125}I counts are found to parallel closely the $A_{280\,nm}$ profile in the sucrose gradient, and SDS polyacrylamide slab gel electrophoretic analysis of such preparations shows that the protein banding patterns obtained by staining with Coomassie blue or by ^{125}I autoradiography are essentially identical. These observations indicate that the reaction labels only a discrete portion of the cellular proteins of intact cells, that this selectivity is dependent on the integrity of the cells and that all of the labelled proteins reside on the outer surface of the plasma membrane where they are accessible to trypsin.

During the first 12–14 h of normal differentiation (after depletion of nutrients) *D. discoideum* proceeds from unicellular amoebae to a multicellular organism composed of ap-

proximately 5×10^5 cells (ref. 14). At any point during these early stages of development the organism can be readily collected and mildly disrupted into a single cell suspension[15]. If intact cells of such samples are labelled with ^{125}I and their isolated membranes analysed by SDS-polyacrylamide gel electrophoresis, the results shown in Fig. 2 are obtained. For convenience, seven of the more noticeable ^{125}I-containing bands are numbered. It is interesting that none of these seven labelled bands (with the possible exception of band No. 6) comigrates with any of the major Coomassie blue staining bands of the same preparations. Although quantitative changes can be seen in several of the labelled proteins, the most noticeable change is the appearance of band No. 4 between 10 and 12 h after plating. At this point in the morphogenetic pathway, *D. discoideum* has just formed firm pointing aggregates. Up to 10 h after plating there is an apparent increase in the labelling of bands Nos 2, 5, and 6; whereas, the labelling of band No. 3 seems to decrease over this period.

The initial triggering event for the shift from the unicellular state to the multicellular state is the exhaustion of nutrient supply[14]. Thus, the zero point for the timing of developmental steps in *D. discoideum* is quite easily established experimentally. Gerisch has shown that the functional state of cells maintained in suspension in a nutrient free medium changes; after 8–9 h they become capable of forming aggregates immediately after plating[1]. These aggregation competent cells, like normal aggregating cells, show an elongated morphology and a preference for end-to-end contacts. Furthermore, they express an antigen(s) which is present on the surface of aggregating cells, but not on growth phase, single cells[4]. As shown in Fig. 3, *D. discoideum* can be maintained in suspension culture for at least 36 h without the appearance of the ^{125}I-labelled band No. 4. The same result is obtained for suspension cultures maintained for shorter periods of time, for example 16 h. It is interesting that in these conditions, as in normal development, the amount of labelling of bands Nos 2, 5 and 6, seems to increase while that of band No. 3 decreases considerably. Moreover, if these same cells are plated to allow them to form normal aggregates, the labelling of band No. 4 appears very rapidly and coincident with the morphological development of firm pointing aggregates.

Thus, in our conditions the acquisition of 'aggregation competence' in suspension cultures is not accompanied by any major qualitative change in the iodinatibility of cell surface proteins, although interesting quantitative changes do occur. Band No. 4 (which appears coincident with the formation of firm aggregates after about 12 h of normal development) is iodinatible only after the suspension culture cells are plated and allowed to form firm aggregates. Consequently, the iodinatibility of band No. 4 does not appear to be associated with the changes in cell surface molecules which are necessary for chemotaxis or aggregation competence in *D. discoideum*. These experiments show clearly that the availability of band No. 4 to this reagent is not due solely to the timing of independent metabolic events initiated by nutrient deprivation. Rather its iodinatibility seems to require participation of the cells in certain intercellular and morphogenetic interaction.

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Meiosis in *Dictyostelium mucoroides*

THE cellular slime moulds are favourable organisms for the study of morphogenetic processes. But so far, genetic analysis of their development has been hampered by the apparent absence of a true sexual cycle, although progress has been made in parasexual genetic analysis[1–3]. Recent ultrastructural observations of the macrocysts of two species have suggested, but not proved, that karyogamy and meiosis are an integral part of dormancy and germination of this developmental stage[4–6]. We present here genetic confirmation that in *D. mucoroides* (strain DM-7) the assortment of mutant traits among progeny of single macrocysts is consistent only with meiosis.

Macrocysts of DM-7 are postulated to contain at least one zygote. The fusion and karyogamy of two haploid cells of possible genotypes (*aB*) and (*Ab*) gives rise to a dihybrid of genotype (*AaBb*). Since DM-7 is homothallic, either parental genotype may fuse with itself as well, giving roughly equal numbers of uniparental and biparental macrocysts, on the assumption that there is one zygote per cyst[7]. In biparental cysts, the assortment of genetic factors among the four primary daughter nuclei of a meiosis results in a particular type of tetrad. Only three kinds of tetrad are possible from a single meiosis, regardless of the linkage relationships of the markers in the dihybrid nucleus[8]. They are the parental ditype ($aB+aB+Ab+Ab$), the non-parental ditype ($AB+AB+ab+ab$), or the tetratype class ($aB+Ab+AB+ab$). DM-7 is advantageous in that single macrocysts can be germinated wtih high efficiency and progeny samplings analysed for possible tetrad assortments..

Individual macrocysts possessing two or more zygotes could possibly arise through independent karyogamies, or by mitoses of a diploid zygote nucleus. These alternative mechanisms are distinguishable from the outcome of a single meiotic event (see below).

The progeny yield of DM-7 macrocysts often approaches 200 amoebae. Ultrastructural studies of germinating cysts have indicated that cytoplasmic cleavage is preceded by many nuclear divisions[6]. The timing of these apparent mitoses relative to a postulated meiotic event may influence the efficiency of this system for genetic analysis.

From these considerations and others, we predict that progeny of a single macrocyst will assort into one of only three tetrad classes, on the assumption that: (*a*) a single karyogamy followed by one (or more) meiosis generates all of the progeny; (*b*) there is no seriously detrimental mitotic or sampling selection against any single progeny

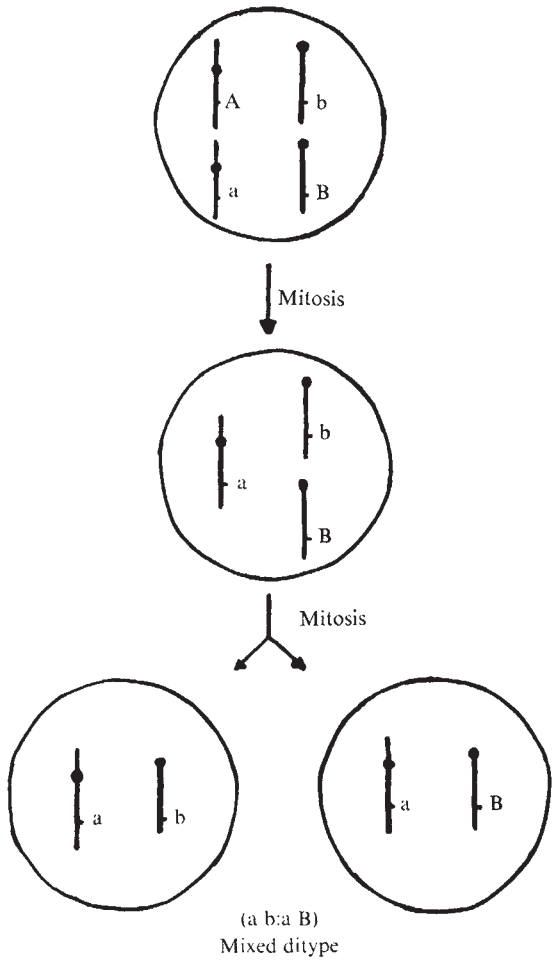

Fig. 1 Diagram of chromosomal losses associated with a hypothetical mitotic gene assortment mechanism in macrocysts. At the top is a dihybrid 'zygote' nucleus shown with only two chromosome pairs for clarity. An intermediate aneuploid stage during haploidisation, and two stable haploid nuclei are also represented. The mechanism of chromosomal loss is not depicted, whether by mitotic non-disjunction or by random cytoplasmic destruction. In the event that all daughter nuclei retain only two assortments of the markers of ditype will result. This outcome is most likely if one of the chromosomes bearing a mutant allele is lost at the first mitosis as indicated here. Otherwise, all copies of it must be lost from all daughter nuclei in successive mitoses. On a random basis six ditypes are equally probable, four mixed (for example, one shown above), one parental ditype and one non-parental ditype. The mixed ditype patterns of assortment do not occur in meiotic reduction division (see text).

genotype, and (*c*) there is no assortment through mitotic haploidisation.

Assumption (*c*) implies the possibility that within macrocysts some sort of accelerated parasexual mechanism could account for assortment patterns. Parasexual diploidy has been induced in *D. discoideum* under conditions similar to those used for macrocyst production[3]. The dihybrid zygote could undergo haploidisation by chromosomal losses in successive mitoses[9]. If such losses are random, single macrocysts would yield predominantly tetratype assortments but also several combinations inconsistent with meiosis (for example, Fig. 1). In the event that two genotypes arise from a single macrocyst, the parental and non-parental ditypes are just two of six equally probable ditype combinations (unlinked markers). So called 'tritypes' involving any three of the four segregant genotypes should also be recovered frequently. Thus recovery of mixed ditypes (Fig. 1) and tritypes together provides a simple test for the occurrence of mitotic haploidisation.

Table 1 Progeny samplings of single macrocysts for the cross *plq* × *cac*

Parental genotypes	Sample progeny clone phenotypes*				Macrocyst tetrad classification†	No. of macrocysts scored
	(*plq*,+)×(+,*cac*)	(*plq*,+)	(+,*cac*)	(+,+)	(*plq*,*cac*)	
	145	0	0	0	PT (*plq*)	2
	0	398	0	0	PT (*cac*)	5
	34	37	0	0	PD	2
	0	0	16	19	NPD	1
	49	72	36	36	TT	6
Totals	228	507	52	55		16

Macrocyst production techniques were slightly modified from those worked out by Nickerson *et al.*[10]. Cells of each genotype were collected and mixed before they became aggregation competent. Aliquots (0.25 ml) containing about 1.0×10^7 cells of each genotype were deposited on 0.1% lactose, 0.1% peptone agar, the same medium as used for growth, but without additional bacteria. They were then placed in metal cans at 21° C to produce macrocysts. After storage for 22–35 d, the cross plates were removed from the container to initiate germination[11]. Single macrocysts were picked and deposited in drops on to fresh, distilled water agar (2%). Drop transfers on the water agar effectively washed the macrocysts free of contaminating spores or amoebae. The germination plates were placed at 13°–15° C in a humid incubator, the interior illuminated by two 40 W incandescent tube bulbs. Significant germination began within 4 d and ultimately reached 60–80% of the transfers. Progeny of germinating macrocysts usually culminated in a small fruiting body on top of the cyst case. Blocks of agar with the cyst case, fruiting body and occasionally amoebal progeny were transferred to small aliquots of Bonner's salt solution[13] containing suspended *E. coli*. The vortexed suspension was spread on to two PDB-5% agar plates per macrocyst and scored for clonal phenotypes after 3 d incubation at 28° C. Double mutant and wild type progeny clones were stable when sub-cloned on PDB-5% agar.

* Genotypes: (*plq*,+) = *plaque*, (+,*cac*) = *cactus*, (+,+) = wild type, (*plq*,*cac*) = the double mutant. The linkage relationships are undefined. The numbers are total progeny for the sum of the macrocysts scored.

† Tetrad classifications: PT, single parental type, PD, parental ditype; NPD, non-parental ditype; TT, tetratype class.

In this study, amoebae or spores were usually cloned on agar plates spread with *Escherichia coli* (B/r) for the screening of mutant strains and for analysis of the products of the genetic crosses. Clonal phenotypes of the three mutants selected for this study are shown in Fig. 2. The mutants *cactus, tag* and *plaque* each have an unusual morphological behaviour in one aspect of either clonal growth, aggregation streaming or fruiting body construction. In pairwise crosses each mutant phenotype is sufficiently distinctive to permit identification of all expected progeny phenotypes.

The crosses were initiated by mixing equal numbers of cells of each genotype under conditions leading primarily to macrocyst formation[10]. Individual macrocysts were germinated according to the method of Nickerson *et al.*[11] Contamination of the germinating macrocyst progeny by non-encysted parental amoebae or spores was eliminated by carefully controlling the conditions of macrocyst production, and after dormancy, by washing the cysts with drop transfers on the germination agar (H_2O). The lactose–peptone agar used for macrocyst production was dried overnight so that when aliquots of mixed cells (0.3 ml) were spread, a liquid film covering the aggregations persisted for only a few hours. Under prolonged wet conditions, the amoebae secrete large amounts of a slime material which traps cells or spores around the maturing macrocysts and prevents effective isolation and washing of the cysts. The complete set of progeny recoveries for the cross of *plaque* and *cactus* (*plq* × *cac*) is presented in Table 1. The numerical distribution of macrocysts classified as tetrads for all the crosses performed is given in Table 2.

The macrocyst progeny of *plq* × *cac* consistently fell into five classes (Table 1), three tetrad types and the two parental classes (selfed macrocysts). The reassortment of

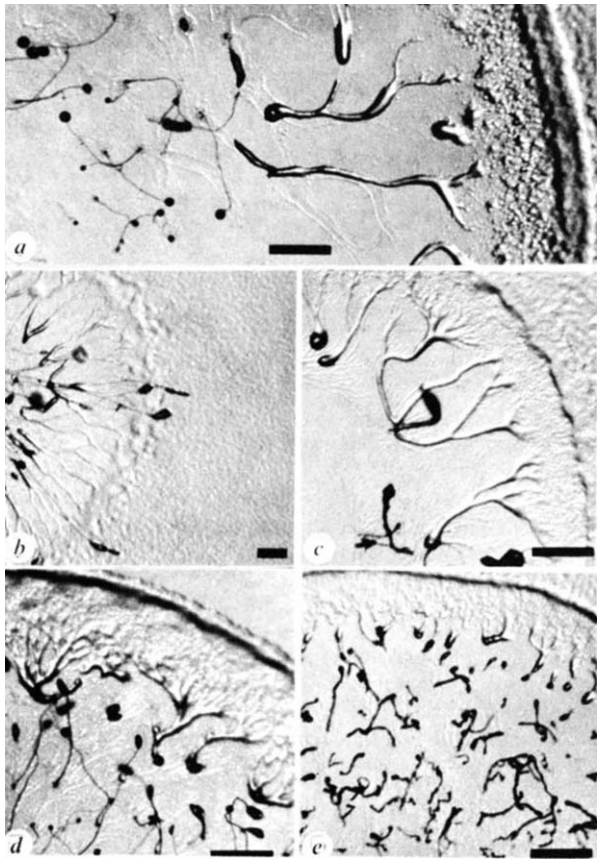

Fig. 2 Clonal morphology of DM-7 wild type and derivative strains (all bars represent 0.5 mm). The wild type stock and mutants were cultured with *E. coli* B/r on a buffered peptone–dextrose agar (designated PDB-2%)[6] at 21° C or 28° C. Mutagenesis procedures followed the method of Yanagisawa *et al.*[12], using nitrosoguanidine (Aldridge Chemical Co.). Surviving cells were initially regrown and screened on PDB with 5% replacing the 2% agar. The increased agar concentration restricts clonal spreading in the wild type (*A*) without affecting morphogenetic behaviour (screening efficiency 100+ clones per plate). *a*, DM-7 wild type. The clone boundary at right is the feeding front of amoebae on the bacterial lawn. Aggregation streams orient radially and are prominent, with complete fruiting bodies at the centre of the clone. *b*, The mutant clone *plaque* (*plq*; isolate 773–2). This mutant has a spreading feeding front on 5% PDB forming a 'halo' appearance around the clone, and prominent streaming, with complete though somewhat fragile fruiting bodies. *c*, Mutant *cactus* (*cac*; isolate 773–18). This mutant has a clone boundary and streaming like wild type, and complete fruiting bodies with excessively thickened stalks. *d*, Mutant *tiny aggregations* (*tag*; isolate 773–23). The clone boundary in this mutant is like wild type but aggregation size is much reduced in proportion to the clone size. Streams are radially polar and fruiting bodies are small, but complete. *e*, *cactus*, *tag*, a double mutant, which has reduced aggregation size characteristic of *tag* while stalk thickening indicates the cactus phenotype.

the two markers to produce wild type and double mutants in seven tetratype and non-parental ditype macrocysts conclusively establishes the existence of a dihybrid zygote stage (possible genotype: *plq*/+, *cac*/+). Second, significant numbers of progeny of each genotype were recovered from these macrocysts. Although the relative numbers are not always statistically equal, the suggestion is strong that there is no detrimental mitotic selection against any one of these genotypes during germination.

The mechanism of gene assortment, either meiotic or parasexual, is established from the data of Table 2. Of the 74 macrocysts germinated in the four crosses, 22 were classified as parental ditypes, non-parental ditypes or tetratypes. Each biparental macrocyst yielded a progeny genotype assortment in one of only the three normal kinds

Table 2 Tetrad classification of individual macrocyst germinations in four crosses

Parental genotypes	Macrocyst tetrad classifications				Total no.
	PT	PD	NPD	TT	
(*plq*,+)×(+,*cac*)	7	2	1	6	16
(*plq*,+)×(+,*tag*)	12	0	0	5	17
(*cac*,+)×(+,*tag*)	11	1	0	4	16
(*cac*,*tag*)×wild type	22	0	1	2	25
Totals	52	3	2	17	74

Genotype and tetrad abbreviations are as in Table 1. The assignment of recombinant phenotypes was not difficult for any of the crosses. To prove the identification of the putative double mutant (*cac*,*tag*) it was back crossed to the wild type stock (line 4 above). Cactus and tag phentotypes were recovered in the single NPD and two tetratype macrocysts germinated. No unexpected progeny phenotypes were observed in any crosses, indicating that each mutant has a single lesion affecting its visible morphology.

of tetrads, as expected for meiotic behaviour. Of particular interest are the ditypes; among 22 biparentals none germinated into the 'mixed ditype' outcomes which in parasexuality would be expected to be recovered with a higher frequency (ratio 2: 1, Fig. 1) than the total number of parental and non-parental ditypes (five germinated). The absence of mixed ditypes argues strongly that parasexual haploidisation is uncommon or not a part of the macrocyst cycle.

An important question about the possible occurrence of multiple meioses is not totally resolved. The data in Table 2 are not consistent with the occurrence of independent karyogamies. In this case, few uniparental macrocysts should arise, and two kinds of expected tritypes ($aB+AB+ab$) and ($Ab+AB+ab$) were not found. Otherwise, mitoses of one original zygote before meiosis is still a possibility. This situation should never produce more than 50% ditypes from biparental macrocysts, whereas, a single meiotic event with no intrachromosomal crossing-over should yield 100% ditypes from the biparentals[8]. We are looking into this question, since any mutants which when crossed produce high frequencies of ditypes will also greatly facilitate the identification of linkage groups and mapping analyses.

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'Isotope effect' in metabolism of radioactive cortisol in man

TRITIUM-labelled steroids have been widely used as tracers in the investigation of steroid metabolism, due to their high specific activity, low cost and low radiation dosage *in vivo*. The validity of any experiment using labelled steroids depends on the fact that the labelled and the endogenous materials are metabolised in a similar manner. In the case of the *in vivo* metabolism of the 1,2-^{3}H-labelled and endogenous cortisol in man, proof of this is lacking. The experiments of Cope and Pearson[1], and of Gold and Crigler[2] using mixtures of 1,2-^{3}H-cortisol and 4-^{14}C-cortisol suggested that these two different species of labelled cortisol were metabolised in a similar manner, and from this it was inferred that they could both be used as valid tracers for studying cortisol metabolism.

In the course of a study of the uptake and metabolism of cortisol by the human forearm[3], we made the preliminary observation that following the intra-arterial injection of a mixture of 1,2-^{3}H-cortisol and 4-^{14}C-cortisol into two healthy men, cortisol isolated from both arterial and venous plasma from one subject had a lower isotope ratio (^{3}H:^{14}C) than the injected mixture. We have now injected intravenously a mixture of 1,2-^{3}H-cortisol and 4-^{14}C-cortisol into six healthy men and have produced substantial evidence of an istope effect.

The two radioactive steroids were stated by the supplier to be at least 96% pure. Dilution analysis immediately before use showed that they were both at least 95% pure. Blood samples were obtained 2 min after the injection and at 30 min intervals thereafter for 4 h. Urine collections were made at 2, 4, 8, 12 and 24 h after the injection. Unlabelled cortisol (100 μg) and unlabelled cortisone (100 μg) were added to each plasma sample (n=48); corticosteroids were extracted using dichlormethane and separated by paper chromatography using the system benzene–methanol–water (2:1:1). The cortisol and cortisone fractions were further purified by paper chromatography using iso-octane–t-butanol–water (10:5:9) and on a thin layer of silica gel using chloroform–methanol–acetic acid (90:5:5). The isotope ratios were measured on the TLC eluates.

The principal findings are summarised in Table 1. Regression analysis shows a significant relationship (at the 0.1% level) between plasma cortisol isotope ratio and time. There were no significant differences between the isotope ratios of cortisol and cortisone.

After hydrolysis of aliquots of urine with β-glucoronidase, the tetrahydro metabolites of cortisol were extracted with ethyl acetate and separated by paper chromatography (benzene–methanol–water; 2:1:1). The eluted metabolites were treated with sodium borohydride and sodium metaperiodate and the resulting 11-hydroxy-17-oxosteroids were chromatographed on paper using toluene–iso-octane–methanol–water (80:120:160:40), and their isotope ratios determined. No significant differences were found in the ^{3}H:^{14}C ratio of 3α,11β-dihydroxy-5β-androstan-17-one (11-HE) derived from 3α,17α,21-trihydroxy-5β-pregnan-11, 20-dione (THE) or from 3α,11β,17α,21-tetrahydroxy-5β-pregnan-20-one (THF), and that of the purified starting material (10.25 $\pm$ 0.35). However, in all 30 urine samples, 3α,11β-dihydroxy-5α-androstan-17-one (11-HA) derived from 3α,11β,17α,21-tetrahydroxy-5α-pregnan-20-one (5α THF) had a lower isotope ratio ($8.93 \pm 0.23, P<0.001$) than 11-HE derived from either THE or THF, or from the starting material.

As the maximum degree of radiochemical impurity in each of the injected steroids was 5%, even the most unfavourable combination of circumstances could only account for less than half of the observed difference in isotope ratio between THE and THF, and 5αTHF. In the case of the plasma cortisol data, the fact that neither chromatographic purification nor a short period (2 min) *in vivo* results in a change in isotope ratio suggests that radiochemical impurities cannot account for the changes in plasma cortisol isotope ratio observed over a period of 4 h.

Gold and Crigler[2], and others, have shown that, in contrast to 4-^{14}C-cortisol, 1,2-^{3}H-cortisol tends to separate from unlabelled cortisol in simple chromatographic systems, a phenomenon that we also have encountered in the present study. These physiochemical findings support the view that the behaviour of 4-^{14}C-cortisol more closely resembles unlabelled cortisol than does the tritium-labelled form. The differences we have observed in the *in vivo* metabolism of the two labelled species may therefore indicate that there are similar differences between the metabolism of unlabelled cortisol and its 1,2-^{3}H-derivative.

While these findings do not necessarily invalidate the use of 1,2-^{3}H-cortisol for tracer studies there may be circumstances where knowledge of this effect could significantly influence the interpretation of results.

Table 1 Isotope ratio of labelled cortisol before and after purification, and after recovery from plasma (mean $\pm$ s.d.)

	^{3}H:^{14}C
Material injected (n = 20)	10.00 $\pm$ 0.25
Material injected after purification as for plasma (n = 13)	10.25 $\pm$ 0.35*
Plasma 2 min after injection (n = 6)	9.95 $\pm$ 0.33
Plasma 4 h after injection (n = 6)	8.95 $\pm$ 0.19*

* Significantly different at the 0.1% level.

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The role of calcium ions in initiating transformation of lymphocytes

TRANSFORMATION of lymphocytes by antigens and by non-specific mitogens (lectins and anti-immunoglobulin sera) is a consequence of the binding of the mitogen to specific receptors on the cell surface[1–3]. The receptors rapidly form clusters ('patches') which may aggregate into polar 'caps'[4] at higher mitogen concentration. These events initiate a series of biochemical changes—enhanced uptake of ions and metabolites[1], increased cyclic GMP[5], and increased metabolism of phosphatidyl inositol and other phospholipids (ref. 6 and V. C. M., M. J. Hayman and M. J. C., unpublished)—which culminate in DNA synthesis and mitosis 48–72 h later. Although the sequence, relative importance

and control of these events is not yet defined, there is evidence for a primary role for Ca^{2+}. *Phaseolus vulgaris* phytohaemagglutinin (PHA) initiates a slow accumulation of Ca^{2+} by lymphocytes[7,8] and its stimulation of DNA synthesis is inhibited by citrate[9], EDTA (ref. 9) or EGTA (ref. 10). The inhibition is reversed by Ca^{2+}. It has also been shown that inhibition by the low concentrations (1.4 mM) of EGTA used did not prevent binding of PHA (ref. 11). Although these results showed Ca^{2+} to be an important factor in initiation of transformation it was not clear whether influx of Ca^{2+} was the only or even the main consequence of the binding of PHA.

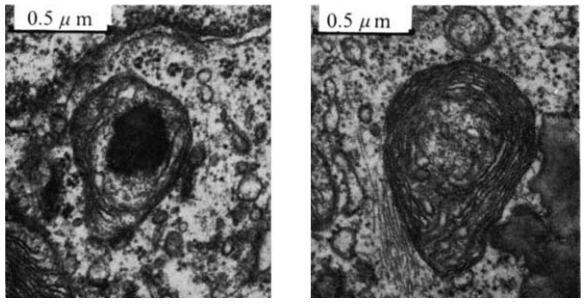

Fig. 1 Mitochondria from pig lymphocytes after 45 h in the presence of the ionophore A 23187 (0.8 μg ml^{-1}). Experimental details of the treatment are given under Fig. 2. In many cells up to one-third of the mitochondria showed dense granules, hypertrophied cristae or both. In dead cells the proportion of these forms was much higher.

Here we show that the Streptomyces antibiotic, A 23187, an ionophore specific for divalent cations[12], initiates a similar series of events to those caused by PHA. The ionophore is a monobasic carboxylic acid (molecular weight 531) two molecules of which form a lipid soluble complex with the cation and enable it to diffuse readily across biological membranes. Its structure has recently been determined[13]. We have studied its effect at micromolar concentration on the morphology of pig lymphocytes and on the incorporation of [6-^{3}H]-thymidine into DNA.

Pig lymphocytes were obtained from mesenteric lymph node as previously described[14]; about 15% of the cells were stained by a fluorescein-conjugated rabbit antiserum to pig immunoglobulin and on the basis of this criterion were judged to be B lymphocytes. Preliminary experiments showed a marked response to low concentrations of A 23187 (0.8 μg ml^{-1} per 10^6 cells). After 45 h culture the cells were indistinguishable under the light microscope from cells incubated with PHA and were agglutinated to a similar extent. Examination of electron micrographs revealed that 80–90% of the cells were enlarged (> 8 μm in diameter) and showed many of the features characteristic of PHA-transformed lymphocytes[15]. Thus, the cytoplasm was enlarged and showed marked endocytotic activity, the plasma membrane showed large pseudopodium-like extensions, there were many polysomes and an increased number of mitochondria. The main differences from PHA-treated cells were a lesser development of the rough endoplasmic reticulum and marked abnormalities of the mitochondria (Fig. 1) including large dense granules and hypertrophied cristae. In contrast with these enlarged cells, about 10% of the ionophore-treated cells appeared in all respects to resemble normal unstimulated lymphocytes and the possibility that A 23187 fails to activate a subpopulation of lymphocytes cannot be ruled out at present.

To obtain a quantitative measure of the stimulus, we followed the uptake of ^{3}H-thymidine into DNA after exposure to A 23187 for 45 h. The response to increasing amounts of ionophore is compared with that to PHA in Fig 2. Both PHA and A 23187 gave a sharp increase in thymidine uptake when their concentrations exceeded a

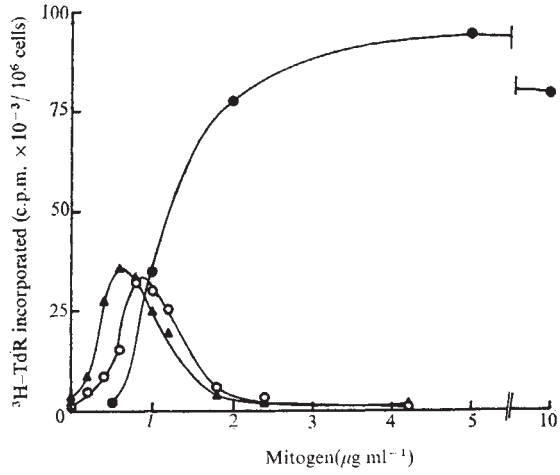

Fig. 2 Incorporation of ^{3}H-thymidine by pig lymphocytes as a function of concentration of A 23187 (○) or of PHA (●). The effect of sub-threshold amounts of PHA (0.3 μg, Wellcome Reagents Ltd) on the response to A 23187 is also shown (▲). The assays were performed as described previously[14], using cells (10^6) from pig mesenteric lymph node in 1 ml of Eagle's medium (containing 1.8 mM $CaCl_2$, 1 mM $MgCl_2$) supplemented with 20% (v/v) foetal calf serum. A 23187 was dissolved in ethanol (1.5 mg ml^{-1}) and diluted with 0.5 volumes of 10 mM Tris HCl buffer, *p*H 7.4; solutions were stored at 2° C in the absence of light and were used up to 4 weeks after preparation. Cultures were incubated at 37° C in an atmosphere of air containing 5% (v/v) CO_2 for 45 h before the addition of ^{3}H-thymidine (1 μCi) and for a further 5 h before recovery of the DNA. The reported values represent the means of duplicate estimations. The PHA curve was obtained with a different batch of cells from that used for the other two curves. The cells used for the A 23187 gave an incorporation of 140,000 c.p.m. with 2 μg PHA (see Fig. 5).

certain critical threshold value and maximal incorporations of thymidine were induced by 0.8–1.0 μg of A 23187 and 2–4 μg of PHA. The response to PHA fell relatively slowly at higher concentrations whereas that to A 23187 declined sharply when the optimal concentration was exceeded. The absolute amounts of radioactivity varied considerably from one batch of cells to another but the maximal stimulation by the ionophore was usually about one-third to one-quarter of that caused by PHA. In view of this morphological evidence in support of transformation, it seems likely that the lower thymidine incorporation induced by A 23187

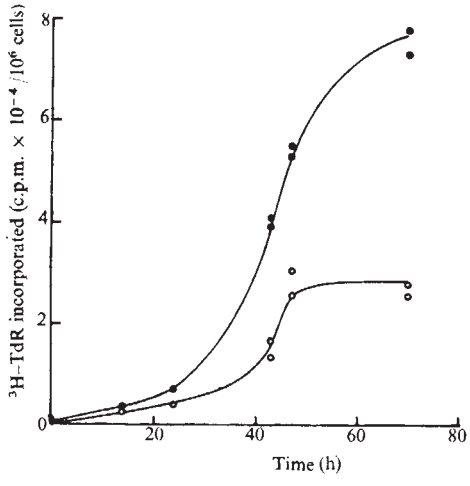

Fig. 3 Time course of ^{3}H-thymidine incorporation by pig lymphocytes in the presence of A 23187 (○) and PHA (●). Assays were performed as previously described using amounts of A 23187 (0.8 μg ml^{-1}) and PHA (2 μg ml^{-1}) giving maximal stimulation. ^{3}H-thymidine was incubated with cultures for a period of 4 h before the cultures were removed and the DNA was recovered.

relative to PHA does not represent a lower proportion of activated cells but is due to the perturbation of the metabolism of the cell by the penetration of the ionophore to the mitochondria.

We have not yet checked whether all the biochemical effects of PHA are duplicated by A 23187, but the parallelism of the morphological responses suggests a similar biochemical basis. Furthermore, the experiment shown in Fig. 3 demonstrates that thymidine uptake follows a similar time course with the two different stimuli.

If both mitogens act by facilitating entry of Ca^{2+} then it should be possible to obtain a response by combining subthreshold amounts of each. The curve (▲) in Fig. 2 shows this effect when the response to A 23187 was measured in the presence of 0.3 $\mu g\ ml^{-1}$ PHA, which by itself had little effect (^{3}H-TdR incorporated: 3,600 c.p.m. 10^6 cells). This result also suggests that largely overlapping populations of cells were involved in the response to both agents. The converse experiment showing PHA response in the presence of A 23187 was complicated by inhibitory effects of the ionophore which will be considered later.

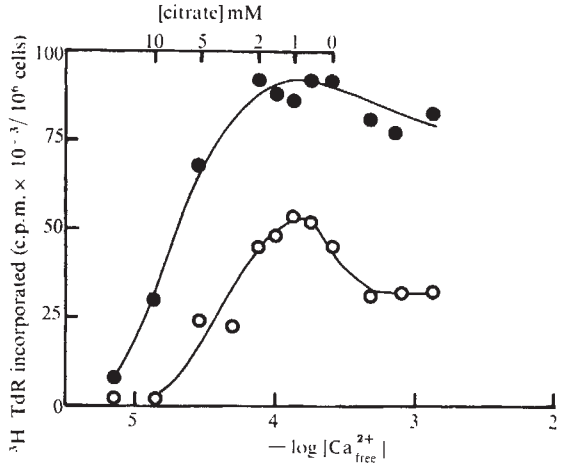

Fig. 4 Effect of concentration of Ca^{2+} on response of lymphocytes to PHA (●) or A 23187 (○). The experiments were carried out in foetal calf serum ($[Ca^{2+}]=3.2$ mM, measured spectrophotometrically using murexide[16]) diluted to 1:10 with Ca^{2+} free Eagle's medium. Ca^{2+} concentration was controlled by addition of Ca^{2+} or sodium citrate. In those samples in which extra Ca^{2+} was added it was assumed that 60% was free, the remainder being bound to PO_4 (1 mM, $K=1.8$ mM) or serum albumin (0.2 mM in Ca^{2+} binding sites, $K=1$ mM[17]). In those samples in which Ca^{2+} was complexed by citrate the concentration of bound divalent cation (x) was calculated from the total $[Ca^{2+}+Mg^{2+}]$ (M) and the concentration of citrate (C) and the dissociation constant $K=$ 0.5 mM, which is almost the same for both ions[18].
$2x=(M+K+C)-[(M+K+C)^2-4MC]^{1/2}$. The fraction of free $Ca^{2+}=(1-(x/M))$.

The critical role of Ca^{2+} in both responses was confirmed by lowering its concentration in the medium (Fig. 4). First, a basic Ca^{2+} free medium was supplemented with 10% (v/v) of foetal calf serum, instead of the usual level of 20%, to give a Ca^{2+} concentration of 0.3 mM. This lowering of Ca^{2+} barely affected the response to PHA but it significantly enhanced the response to the ionophore. Further lowering of Ca^{2+} was effected by addition of sodium citrate at concentrations between 1 mM and 10 mM. The results in Fig. 4 show that there was a sharp drop in response to A 23187 when $[Ca^{2+}]$ fell below 10^{-4} M, close to the dissociation constant of the complex between Ca^{2+} and A 23187[19]. The response to PHA fell when a slightly lower Ca^{2+} concentration was reached (half maximal response at 2×10^{-5} M) in good agreement with the results of Alford[9] and of Whitney

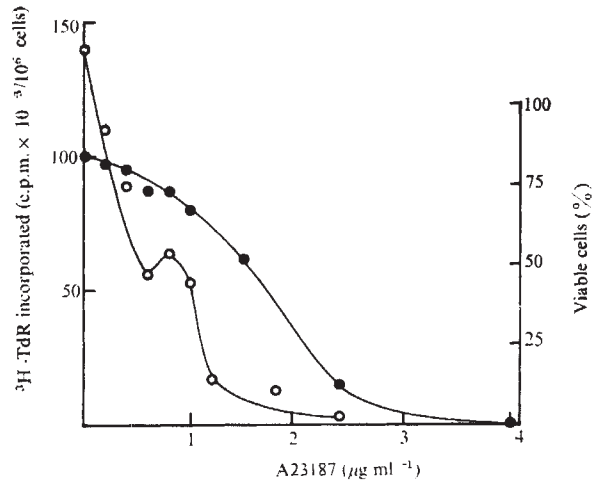

Fig. 5 Effect of A 23187 on incorporation of ^{3}H-thymidine into pig lymphocytes stimulated by 2 μg PHA per 10^6 cells (○) and the viability of the cells (●). The experimental procedure is given under Fig. 1. PHA and A 23187 were both added at the beginning of the experiment. The viability after 45 h of incubation with A 23187 was measured as the percentage of cells which excluded trypan blue.

and Sutherland[10]. We conclude that A 23187, like PHA, requires Ca^{2+} in the external medium in order to be effective. Addition of a 1 mM excess of Ca^{2+} over citrate (5 mM and 10 mM) restored the response both to PHA and to A 23187 to the level of the controls.

The inhibitory effects of A 23187 on the response to PHA are shown in Fig. 5, together with a curve showing the decrease in viability of the cells. Although the two curves show a rough correspondence it is clear that there is initially a marked inhibition without significant loss of viability and that later, above the normal threshold for stimulus by A 23187, an activating effect is superimposed on the declining incorporation of thymidine. It is not easy to explain this activation if a single population of cells, already fully stimulated by the PHA, is involved, unless A 23187 has some effect in addition to facilitating the influx of Ca^{2+}. Another explanation is that A 23187 can stimulate a subpopulation of lymphocytes which are not affected by the PHA. This would be consistent with the high proportion of cells which enlarge under the influence of the ionophore. Experiments to distinguish between these possibilities are in progress.

It is likely that much of the toxic effect of the ionophore can be explained in terms of its effects on the mitochondria, the morphology of which is grossly disturbed. It is well known that mitochondria have a large capacity for taking up Ca^{2+}, especially in the presence of phosphate and other permeant anions[20] and it is also known that A 23187 stimulates this uptake and diverts the energy from both respiration and from exogenous ATP into Ca^{2+} transport[16]. As inhibitors of glycolysis and of respiration are known to block transformation[21] it is not surprising that A 23187 is also inhibitory. Mouse and rat spleen cells were more sensitive than pig lymphocytes to the toxic effects of the ionophore, only 20% surviving after 40 h at 0.8 $\mu g\ ml^{-1}$, and we have been unable to produce significant stimulation of thymidine uptake by these cells.

Except for its inhibitory effects, A 23187 produces a very similar response in pig lymphocytes to that produced by PHA. In addition to the results described above we have preliminary evidence which shows a stimulus of phosphatidyl inositol turnover and an inhibition of PHA stimulated phosphatidyl inositol turnover by citrate. If the effect of the ionophore is limited to its facilitation of divalent cation

movements then those of PHA can be similarly explained. We therefore suggest that lymphocyte transformation is the result of a direct effect of the mitogen on the permeability of the plasma membrane to Ca^{2+}.

Two interesting questions follow. First, how does PHA increase permeability to Ca^{2+} and, second, what are the further effects of Ca^{2+} inside the cell?

The most striking common feature of the action of a variety of mitogens on lymphocytes is the requirement for multivalency[1,3]. This generalisation also applies to the release of histamine from mast cells by antibodies to IgE, a response which can also be induced by A 23187 in the presence of Ca^{2+} (ref. 22). If the receptor proteins bridge the lipid regions of the membrane then it is plausible to suggest that clusters of receptors might contain polar channels permitting the passage of Ca^{2+} and possibly of other ions into the cell. This may be but one example of a general mechanism for transmitting signals across a membrane following the interaction between a specific receptor and the appropriate stimulus.

Many intracellular processes are controlled by the concentration of Ca^{2+} (ref. 23); at least three of these are involved before DNA synthesis in stimulated lymphocytes. Activation of phosphorylase kinase[24] is required for the initiation of glycolysis[21]; the microfilament–microtubule system is required for endocytosis[25] and its inactivation blocks transformation[26]; lastly and most significantly, the level of cyclic GMP in some cells has been shown to be controlled by Ca^{+2} (ref. 27) and its concentration in lymphocytes rises immediately in response to PHA (ref. 5). The further effects of cyclic GMP are not yet defined but in other tissues it has been implicated in several hormonal responses[28] and it is clearly an important intermediary.

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Actions and interactions of colchicine and cytochalasin B on contraction of granulation tissue and on mitosis

IT has been shown by Majno *et al.*[1] that a strip of growing granulation tissue (GT) cut from the wall of a 2- to 4-week-old granuloma pouch[2] or from the base of an open wound in the rat, contracts *in vitro* in response to 10^{7}–10^{6} concentrations of 5-hydroxytryptamine, angiotensin and other agonists. An association between this pharmacological reactivity of GT and the phenomenon of wound contraction has been proposed[1] on the bases that the forces which cause a wound to contract are cellular in origin and generated by GT cells[3,4], and that fibroblasts proliferating *in vitro* and *in vivo* develop a muscle-like cytoarchitecture characterised by an increase in the number of microfilaments[5]. Also fibroblasts in culture contain abundant microtubules[6] which can be rapidly destroyed by poisons such as colchicine[6-8] that prevent polymerisation of the tubular protein. For example colchicine can prevent microtubules comprising the mitotic spindle from forming and thereby inhibit karyokinesis (separation of chromosomes)[9]. The drug seems not to affect the functions of microfilaments, on which various types of contractile activity, including cytokinesis, depend[10]; the microfilaments are specifically inhibited by the drug cytochalasin B (ref. 11).

We performed a series of experiments using the technique of Majno *et al.*[1], primarily to determine the time of onset of contractility of GT after wounding and the effects of X irradiation on contractility. We found that when colchicine or other spindle poisons (vincristine, vinblastine or podophyllotoxin) were added to GT in the bath in 10^{-7}–10^{-6} final concentrations, there slowly developed a marked contraction of the tissue, which was shortened by as much as 30–40% of its initial length (Fig. 1*a*). Contraction induced by colchicine was dependent on the presence of oxygen and was inhibited by 10^{-4}–10^{-5} 2, 4-dinitrophenol (DNP) (Fig. 1*b*). Contraction of GT induced by colchicine and related drugs was also reduced by 10^{-5} KCN and by 10^{-4} papaverine. After contraction of GT had been induced by colchicine, the tissue failed to relax after five or more successive washes with fresh Tyrode solution, which did not free the tissue of the drug. ATP caused GT to contract and DNP caused relaxation. Colchicine failed to cause contraction of GT in the presence of DNP; when the tissue was washed, DNP but not colchicine was removed and the tissue recontracted at the same rate as before the addition of DNP (Fig. 1*b*). It was more difficult to reverse the action of papaverine on contraction caused by colchicine by washing in this way.

When 10^{-7}–10^{-6} cytochalasin B (dissolved in DMSO[11]) was added to GT, the tissue relaxed slightly; subsequent washing restored the initial base line and the preparation then contracted in the usual way on addition of colchicine. When 10^{-6} cytochalasin B was added to the bath, followed (without washing) by 10^{-6} colchicine, the tissue failed to contract, until it was washed with fresh Tyrode solution; this removed cytochalasin B as it removed DNP, and allowed contraction to occur. When cytochalasin B was added after contraction had been induced by colchicine, the tissue relaxed completely (Fig. 1*d*); further washing caused contraction to re-occur, at the same rate as prior to the addition of cytochalasin B in the same way as shown for DNP (Fig. 1*b*).

This reactivity of GT to spindle poisons, metabolically dependent on the presence of oxygen and on oxygen-coupled phosphorylation, and antagonised by cytochalasin B, was shown by strips of GT cut from 7–35-d-old pouches in the rat. We have been unable to demonstrate any pharmacodynamic activities of strips of uninjured subcutaneous connective tissue removed from rats by adding colchicine, cytochalasin B, 5-hydroxytryptamine, angiotensin, histamine or other agonists. But colchicine caused oxygen-dependent contractions, also inhibited by DNP and cytochalasin B, of rat stomach fundus and of rat and guinea pig ileum, similar to GT.

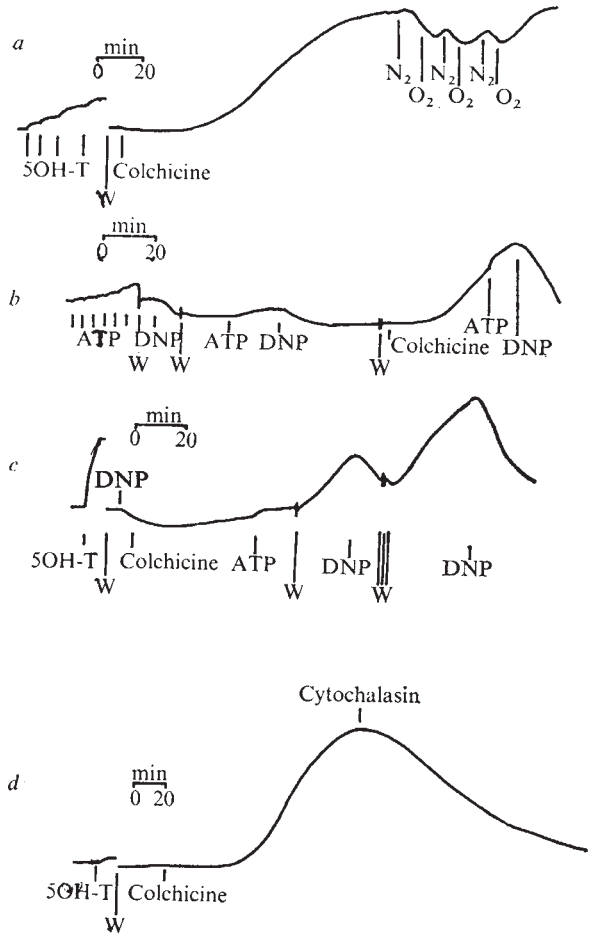

Fig. 1 Reactions of strips of GT (12–14 cm long, weighing 3–6 g and containing 4–5 mg DNA per g wet weight) to drugs were tested in Tyrodes solution at 32° C in 100 ml capacity bath gassed with 95% CO_2/5% O_2, as described by Majno *et al.*[1]. Traces were obtained on smoked paper advancing at 2 mm min^{-1} using a 3:1 lever magnification. Drugs were added in 0.2–0.5 ml aliquots, and the final drug concentrations in the bath expressed as g ml^{-1}; at W tissue washed with fresh Tyrodes solution. *a*, Contractions induced by cumulative additions[15] of 1, 5, 10 and 50 ($\times 10^{-7}$) 5-hydroxytryptamine (5-OHT) and 10^{-6} colchicine; reversible inhibition of latter by nitrogen (N_2). *b*, Contractions induced by additions of 1, 2, 3, 5, 6 and 10 ($\times 10^{-5}$) adenosine triphosphate (ATP); relaxation of tissue was induced by 10^{-5} 2,4-dinitrophenol (DNP) which also inhibited the contraction due to 10^{-6} colchicine. *c*, Shows GT failed to contract to 10^{-6} colchicine in the presence of 10^{-5} DNP until the tissue was washed with fresh medium which removed DNP and caused recontraction due to colchicine. *d*, Contractions induced by 10^{-6} colchicine inhibited by 10^{-5} cytochalasin B. (DMSO used as solvent for cytochalasin B had no effect on contraction of GT.)

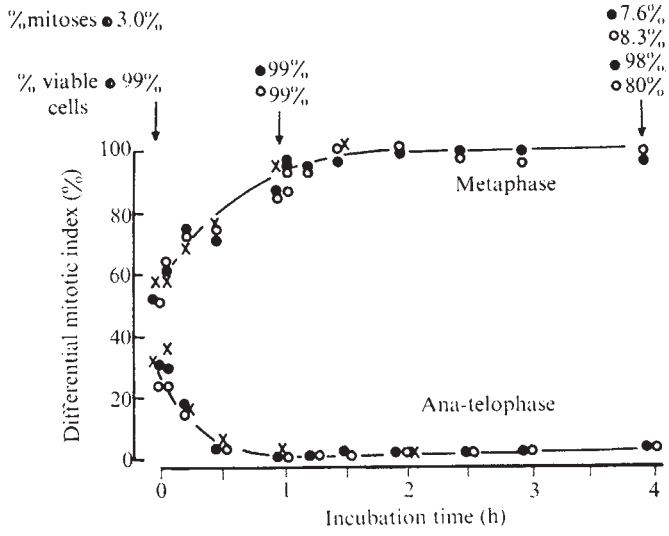

Fig. 2 Metaphase arrest induced by 10^{-6} colchicine in freshly collected W-256 cells (~ 10^6 cells ml^{-1}) incubated at 36° C in medium 199 plus 10% horse serum and gassed with 95% O_2/5% CO_2 (●) or N_2 containing < 3 p.p.m. O_2 (○). The presence of 10^{-5} cytochalasin B failed to influence metaphase arrest induced by 10^{-6} colchicine under aerobic conditions (×). Percentage viable cells determined using nigrosin exclusion technique; total mitotic counts based on all mitotic stages present in 500–1,000 cells per sample; differential mitotic counts based on 100 metaphases counted in each aliquot removed after 0–4 h incubation time.

We measured the effect of colchicine and cytochalasin B under aerobic and anaerobic conditions on mitotic division of W-256 rat tumour cells cultured *in vitro*, which had been collected from ascites fluid when the tumour was at an exponential phase of growth. Differential mitotic counts were made on cell aliquots removed from the cultures at intervals after the drug(s) had been added. Two telophase stages were scored: cytoplasmic cleavage absent (1), and present (2) to establish cytokinesis. During incubation, the cells were bubbled with either 95% O_2/5% CO_2 or with pure N_2 (containing < 3 p.p.m. O_2). Colchicine caused metaphase arrest irrespective of the presence or absence of oxygen or cytochalasin B (Fig. 2, Table 1). But 10^{-5}–10^{-4} DNP, greatly reduced the effect of colchicine on cell division. Cytochalasin B inhibited cytokinesis. Neither the absence of oxygen nor the presence of papaverine affected karyokinesis and cytokinesis significantly, but cytochalasin B was significantly less effective in preventing cytokinesis when oxygen was absent or DNP present (Table 1).

Assuming that colchicine and cytochalasin B act specifically on microtubules and microfilaments respectively, the results obtained indicate that both organelles participate in the maintenance of 'tone' of the contractile cells which develop in GT and are present in stomach and ileum. It is suggested that in cell contraction a microtubular apparatus functions primarily by regulating the tone of the contractile component (microfilaments); that the destruction of microtubules by colchicine releases the microfilaments from the control exercised by the microtubules and indirectly causes contracture to develop, provided oxygen is present and coupled to phosphorylation; and that cytochalasin B inhibits contracture by a specific direct action on microfilaments.

The intracellular movements which take place during mitosis are attributed to successive changes occurring in microtubules (karyokinesis) followed by microfilaments (cytokinesis). The results obtained indicate that neither karyokinesis

Table 1 Differential counts of mitoses in W-256 cells incubated for 100 min in presence of colchicine and other compounds under aerobic or anaerobic conditions

Final concentration of compound (g ml^{-1})	Gas phase	Total mitoses	Differential mitotic count* % P	M	A	T 1	T 2	Significance†
Nil	Oxygen	247	13	40	7	10	30	
Nil	Nitrogen	188	9	52	4	7	28	
10^{-7} colchicine	Oxygen	120	8	84	2	1	5	
10^{-6} colchicine	Oxygen	118	8	85	1	0	6	
10^{-6} colchicine	Nitrogen	113	3	89	2	2	4	
2×10^{-5} cytochalasin B	Oxygen	255	9	39	7	43	2	*a* $P < 0.01$
2×10^{-5} cytochalasin B	Nitrogen	184	2	54	10	24	10	
2×10^{-5} cytochalasin B plus 10^{-6} colchicine	Oxygen	106	4	94	1	1	0	
10^{-4} papaverine	Oxygen	216	8	47	4	6	35	
Nil	Oxygen	207	14	49	5	8	24	
10^{-5} 2, 4 DNP	Oxygen	217	9	46	4	5	36	
10^{-4} DNP	Oxygen	177	18	57	1	3	21	
10^{-5} 2, 4 DNP plus 10^{-7} colchicine	Oxygen	193	9	60	3	5	23	
10^{-4} 2, 4 DNP plus 10^{-6} colchicine	Oxygen	161	7	63	2	3	25	*b* $P < 0.001$
10^{-6} colchicine	Oxygen	113	6	90	0	1	3	
2×10^{-5} cytochalasin B	Oxygen	198	11	51	5	30	3	*c* $P < 0.01$
10^{-4} 2, 4 DNP plus 2×10^{-5} cytochalasin B	Oxygen	219	11	46	2	25	16	

Freshly collected W-256 cells in log growth phase were incubated in medium 199 (plus 10% horse serum) at 36° C. Approximately 10^6 cells were added per ml of medium which had been prewarmed and gassed for 20 min with 95% O_2/5% CO_2 or N_2 before and after addition of the various compounds followed by cells. After incubation, the cells were fixed by adding an excess of methanol–acetic acid (3:1 v/v) and were then stained with acetic orcein. Differential mitotic counts are based on 100 metaphases counted in each preparation.

*Abbreviations: P, prometaphase; M, metaphase; A, anaphase; T, telophase (cytoplasmic cleavage absent (1) or present (2)).

†Based on χ^2 test for T(2)/(A + T1 + T2) in *a* and *c* and for (A + T1 + T2) in *b*.

nor inhibition of spindle synthesis by colchicine require the presence of significant concentrations of oxygen; the considerable energy required for mitosis seems to be supplied from reservoirs built up progressively during the whole cell cycle[12]. It has been found that a slight increase in oxygen consumption occurs at cytoplasmic cleavage of sea urchin eggs[12,13]. We found that anoxia maintained for 100 min caused a 12% decrease in the proportion of type 2 telophase in W-256 cells but this decrease was not statistically significant. DNP caused no significant delays in the progression of the cells through mitosis, but inhibited the actions of colchicine on karyokinesis and of cytochalasin B on cytokinesis. This suggests that uncoupling of phosphorylation by DNP affects active transport of colchicine or possibly its binding to the target protein (microtubule) of the dividing cell. This binding is, however, reversible and does not involve a chemical modification of colchicine[7]; it also possibly affects transport and binding of cytochalasin to the contractile microfilaments. Ultrastructural studies have provided some evidence that microtubule subunits may aggregate to form 100 Å filaments[14].

A comparison of the effects of colchicine, cytochalasin, DNP and oxygen on cell division and on contraction of GT shows that these two cellular activities react similarly. Our findings suggest that microtubules are closely integrated with microfilaments in regulating both these processes. The termation of mitosis in telophase may reflect a loss of control of the tubular component over the microfilaments which causes a spontaneous contracture of the latter and results in cell cleavage, in the same way that inactivation of the corresponding tubular component (by colchicine) in the contractile cell produced contracture. Contracture induced by colchicine, and other spindle poisons, was all-or-none in character; it was not possible to regulate the degree of tissue shortening by altering the drug concentration. This is the type of response which would be caused by a drug which destroys the mechanism on which it acts. Cell cleavage appears to be a phenomenon with similar all-or-none characteristics.

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Reversibility of morphine tolerance and dependence in guinea pig brain and myenteric plexus

WHEN guinea pigs are exposed continuously to high morphine concentrations, tolerance and physical dependence develop within a few days in both the central nervous system (CNS) and the myenteric plexus. In the plexus morphine tolerance is associated with subsensitivity to the inhibitory catecholamines and supersensitivity to the excitatory neurotransmitter serotonin (5-hydroxytryptamine, 5-HT)[1,2]. We have investigated the following questions about these phenomena. (*a*) After opiate withdrawal in animals made tolerant and dependent, would the altered sensitivities to neurotransmitters in the plexus return to normal? (*b*) If so, would loss of tolerance to morphine in the plexus

proceed in parallel with loss of subsensitivity to catecholamines and loss of supersensitivity to 5-HT? (*c*) Would such reversion to normal in the plexus be concurrent with disappearance of CNS indications of tolerance and dependence?

Male guinea pigs (400–450 g) were rendered tolerant and dependent in 3 d by implanting four morphine pellets (75 mg morphine base each) subcutaneously[3]. The pellet residues were removed on the third day after implantation and the animals were maintained for 7 d thereafter. Implantation and removal were carried out under light ether anaesthesia.

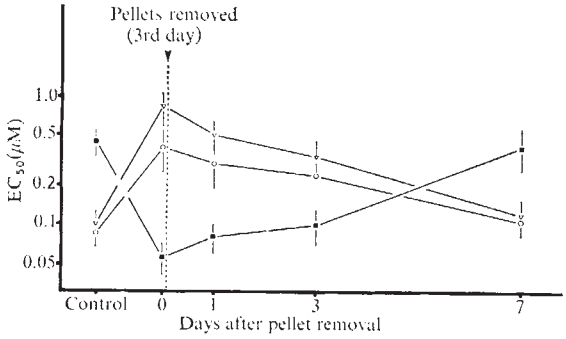

Fig. 1 Changes in sensitivity to morphine (▽), adrenaline (○), and 5-HT (■) of the longitudinal muscle-myenteric plexus preparation of guinea pigs the third day after implantation with four morphine pellets (75 mg morphine base each) and after abrupt withdrawal by pellet removal (third day after implantation). The mean effective dose (EC_{50})± s.e.m. of each drug is plotted against time on a logarithmic scale. Each point is based on four to five animals (eight to ten strips).

The longitudinal muscle strip with attached myenteric plexus from the ileum was prepared according to Rang[4] and mounted in a 5-ml tissue bath as described previously[1]. The bath contained Krebs bicarbonate Ringer solution (37° C) and was bubbled with 95% O_2/5% CO_2. Electrical field stimulation for maximal twitch tension was applied at 80 V, 0.1 Hz and 0.5 ms pulse duration by means of a Grass S4E stimulator. Strips were equilibrated for 1 h with electrical stimulation and washed at intervals of 5–10 min. A force displacement transducer (Grass FT 0.03C) connected to a Grass 7B polygraph recorded the isometric twitches (2–4 g tension). Resting tension was adjusted to 1 g.

Longitudinal muscle strips of untreated animals were prepared after overnight starvation. Morphine-treated animals were killed, and strips were obtained on the third day after pellet implantation, and on the first, third and seventh day after pellet removal. When morphine or catecholamines were tested, electrical stimulation was applied continuously. The drug was washed out after a maximal effect was established. Excitatory compounds, like acetylcholine and 5-HT, were added to the bath 10 s after stopping electrical stimulation. Drugs were added to the bath at intervals of 10–45 min. The concentration required for morphine or catecholamines to inhibit electrically induced twitches by 50% is called EC_{50}. The same term is used for induction of 50% of the preceding electrically stimulated tension by acetylcholine or 5-HT.

Tolerance in guinea pigs was measured by the effect of intravenously injected morphine sulphate (50 mg free base ml^{-1}) on the body temperature. In controls, 30 min after intravenous injection of 10 mg kg^{-1} morphine, a maximal degree of hypothermia was observed, using a thermistor probe inserted 5 cm into the rectum for 2 min. Physical dependence was tested by precipitation of jumping behaviour on intraperitoneal injection of naloxone hydrochloride (50 mg ml^{-1}), as previously described for guinea pigs[1]. Each animal was tested only once.

Figure 1 summarises the responses to morphine, adrenaline, and 5-HT of longitudinal muscle–myenteric plexus preparations isolated from guinea pigs during and after exposure to morphine. After removal of the pellets, tolerance to morphine was gradually lost, as indicated by the decrease of the effective concentration (EC_{50}) required for 50% inhibition of electrically induced twitches. Within 7 d the tissue had recovered normal sensitivity to the opiate (EC_{50}=100 nM).

A similar time course of recovery was found for adrenaline, with virtually normal sensitivity restored by the seventh day after pellet removal.

Sensitivity to the excitatory effect of 5-HT followed a very similar but inverse course. Supersensitivity reached about eight-fold (EC_{50}=55 nM) compared with the non-tolerant preparations (EC_{50}=420 nM). The first day after pellet removal the strips were still six times more sensitive to 5-HT than controls, and even on the third day some supersensitivity persisted (EC_{50}=100 nM). As described for the other drugs, normal sensitivity was established by the seventh day (EC_{50}=400 nM).

The dose-response curve for dopamine inhibition in morphine tolerant strips was very flat, with only 20% inhibition at 100 μM (Fig. 2). The same results were obtained the first day after pellet removal; and even after 3 d, 100 μM dopamine only depressed the twitch tension 40%. Nevertheless, normal sensitivity had returned within 7 d (EC_{50}=10 μM).

The drugs used in the above experiments produce a common effect; they influence the output of acetylcholine induced by electrical stimulation or by 5-HT. Since acetylcholine added to the bath stimulates the longitudinal muscle directly[5], the response to this neurotransmitter may indicate whether or not chronic exposure to morphine affects the sensitivity of the muscle. We noticed no change in sensitivity to the excitatory effect of acetylcholine in strips prepared before or after pellet removal as compared with controls (EC_{50}=0.4 nM). Furthermore, no change in twitch tension induced by maximal electrical stimulation was seen at any time in these experiments.

To determine tolerance in the CNS, the hypothermic action of morphine was measured 30 min after intravenous injection. A dose of 10 mg kg^{-1} caused a fall of rectal temperature of 0.8° C in controls (Table 1). The same dose,

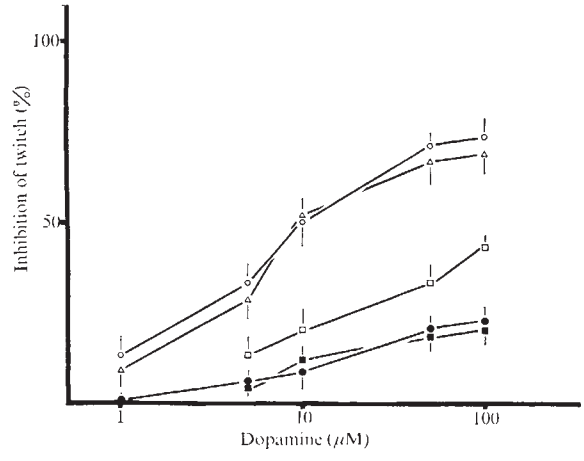

Fig. 2 Dose-response curves for dopamine inhibition of the electrically stimulated muscle twitch. Control (○) strips, preparations from guinea pigs 3 d after pellet implantation (■), and the first (●), third (□), and seventh (△) day after pellet removal. Each point represents the mean depression (±s.e.m.) of the electrically stimulated twitch (N=4-5). For further explanation, see Fig. 1.

3 d after pellet implantation, depressed the temperature by only 0.3° C. After pellet removal, there was no change in the degree of tolerance after 1 d, partial recovery by the third day and complete recovery by the seventh day.

Physical dependence disappeared at approximately the same rate as tolerance to hypothermic effects. As Table 1 shows, after pellet removal naloxone became gradually less effective in precipitating withdrawal jumping; and after 7 d, no jumping at all was observed, even with doses up to 128 mg kg^{-1}.

The results reported here demonstrate that pharmacological alterations observed during morphine tolerance and dependence in the guinea pig myenteric plexus as well as in the CNS are completely reversible. Within 7 d following abrupt withdrawal, sensitivities to morphine, naloxone, adrenaline, dopamine and 5-HT returned to normal. Thus, these findings support the view that opiate tolerance and dependence can disappear rapidly and completely[6-10]. By the methods used here we were unable to observe any persistence of tolerance or dependence[11-14], or of the associated abnormal sensitivities to neurotransmitters.

Table 1 Effect of morphine pellet implantation and removal on tolerance and physical dependence in guinea pigs

	Temperature fall*	N	P†	Naloxone ED_{50}‡ (mg kg^{-1} ± s.e.m.)
Control	−0.80 ± 0.14	5	—	>100¶
Third day after pellet implantation	−0.30 ± 0.05	6	<0.01	2.4 ± 0.9
First day after pellet removal§	−0.32 ± 0.13	6	<0.01	3.2 ± 1.2
Third day after pellet removal	−0.48 ± 0.07	5	<0.01	41.0 ± 3.7
Seventh day after pellet removal	−0.89 ± 0.14	5	N.S.	>128¶

Data show the effect of morphine (10 mg kg^{-1} intravenous) on the rectal temperature and the ED_{50} of naloxone hydrochloride (intraperitoneal) required to precipitate withdrawal jumping.
*Mean °C ± s.e.m., measured at room temperature 20°C.
†Two-tailed *t* test for difference from control.
‡The median effective dose (ED_{50}) ± s.e.m. consists of three to four independently calculated ED_{50} values according to Dixon[18]. Each independent ED_{50} is based on three to four animals.
§Pellet removal on third day after implantation.
¶This dose did not evoke jumping.

Our data show that the time course of recovery after morphine withdrawal is very similar in the myenteric plexus and CNS. This parallelism supports the likelihood of a common underlying mechanism in both nervous tissues. Furthermore, the results obtained in the plexus are in accord with our earlier findings that morphine tolerance is accompanied by supersensitivity to the excitatory neurotransmitter 5-HT[1,2], as first hypothesised by Collier[15]. As discussed[16], this supersensitivity could be a sufficient condition for both opiate tolerance and dependence. The supersensitivity is seen as an adaptive response to chronic inhibition by morphine of nerve pathways containing at least one serotoninergic synapse. Then more morphine would be required to inhibit (that is, tolerance), and a certain amount of morphine would be required to normalise the supersensitive synapses (that is, dependence). When exposure to morphine ends and the ambient morphine concentration declines, the disinhibition of these synapses could account for the abstinence syndrome. In support of the hypothesis is the finding that cyproheptadine, a specific 5-HT receptor blocker in the CNS, abolishes most withdrawal phenomena[17]. The time course of the loss of supersensitivity to 5-HT after morphine withdrawal, which corresponds to the rate of disappearance of tolerance and dependence in the CNS, may reflect the turnover rate of 5-HT receptors.

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Isolation of photoreceptor and conventional nerve terminals by subcellular fractionation of rabbit retina

LITTLE is known of retinal transmitter substances although acetylcholine, dopamine, 5-HT, γ-aminobutyric acid (GABA), glutamate, and aspartate have all been put forward as candidates for such a role[1-9]. In other areas of the central nervous system (CNS), the preparation of isolated nerve-ending particles (synaptosomes) has been extremely valuable in studying central transmitter substances[10-13]. As far as we are aware, however, subcellular fractionation procedures have not previously been applied to the study of retinal transmitter substances.

Here we have subjected the rabbit retina to homogenisation and subcellular fractionation[12,14] and have found that these procedures produce synaptosomes, not only from the conventional type of retinal nerve ending, but also from easily identifiable pinched-off photoreceptor terminals. We have also studied the subcellular distribution in the retina of some endogenous amino acids and of some marker enzymes, particularly those concerned with GABA metabolism. The distribution of exogenously accumulated ^{14}C-GABA and ^{3}H-dopamine was also examined[15]. The results of these experiments support the suggestion that aspartate may be a photoreceptor transmitter and that GABA and dopamine may be transmitters at some of the conventional retinal synapses.

Rabbit retinae were homogenised in 0.32 M sucrose and a crude nuclear pellet (P1) and a crude mitochondrial

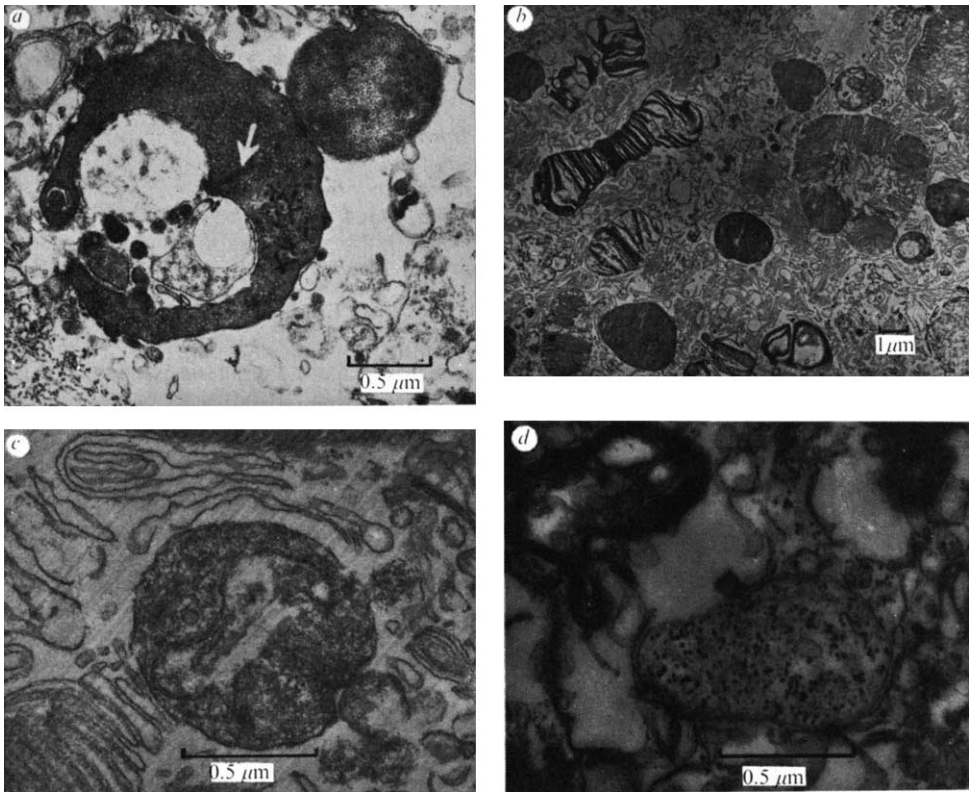

Fig. 1 Electron micrographs of retinal subcellular particles. *a*, Rod photoreceptor terminal: The arrow points to the synaptic ribbon, paired invaginations lie one on each side of the ribbon. Between the ribbon and the invaginations is the arciform density. A sheet of synaptic vesicles lie apposed to the ribbon. *b*, Fragments of photoreceptor outer segment; *c*, synaptosome from a conventional retinal synapse, *d*, particle possibly originating from glia and containing many small electron opaque granules but no synaptic thickening.

pellet (P2) (ref. 12) were obtained by differential centrifugation. For electron microscopy the P1 and P2 pellets were fixed in glutaraldehyde, post-fixed in osmium tetroxide and embedded in Epon–araldite. Sections were stained with methanolic uranyl acetate and aqueous lead citrate and examined by electron microscopy.

The crude nuclear fraction (P1) contained many nuclei, tissue fragments, and a few large mitochondria. Aggregations of smaller mitochondria were frequently seen and these appeared to be derived from sections of photoreceptor inner segments being sheared-off intact during the homogenisation process. In addition, fragmented outer segments were frequently observed. The most striking feature of this fraction, however, was the presence of large numbers of pinched-off rod photoreceptor terminals (Fig. 1*a*). These structures were identified by the presence of densely staining cytoplasm, many synaptic vesicles and a synaptic ribbon with an associated halo of synaptic vesicles. Most of these rod photoreceptor terminals, (diameter 1–2 μm) were sectioned in a plane which revealed the invaginations into which processes from horizontal and bipolar cells normally project[16,17]. Occasionally a much larger cone terminal (diameter 5–10 μm) with less dense cytoplasm was observed. A few nerve endings with light staining cytoplasm (diameter approximately 1 μm) and synaptic ribbon, but no invaginations, were observed in this fraction; these were identified as bipolar terminals.

The crude mitochondrial fraction (P2) contained myelin fragments from the optic nerve, fragmented and partially intact photoreceptor outer segments (Fig. 1*b*), and many free mitochondria. Many conventional nerve ending particles (Fig. 1*c*) from horizontal and amacrine cells were observed, but no pinched-off photoreceptor terminals were identified in this fraction. Bipolar cell terminals containing synaptic ribbons were observed. Many other particles were present which varied in size and shape: these contained numerous small electron-opaque granules (ribosomes) but no mitochondria (Fig. 1*d*) or synaptic thickenings and may have been derived from processes of the neuroglial Müller cells[18].

This preliminary morphological study of the subcellular particles produced by homogenisation of the rabbit retina shows that conventional retinal nerve endings form synaptosomes similar to those previously described for many other areas of the CNS. The photoreceptor terminals, however, are unique in their distinctive morphology and like cerebellar mossy fibre nerve endings[12], but unlike other nerve ending particles, sediment in the P1 pellet. Thus, it should be possible to obtain, for the first time, a preparation containing only isolated photoreceptor terminals. Such a preparation should be of great help in identifying the transmitter substance of the photoreceptors.

It has been proposed that an excitatory transmitter is continuously released from photoreceptors in the dark,

Table 1 Distribution of endogenous amino acids in primary fractions

	% of total recovered in P1 + P2 + SNT.			Relative specific activity (RSA)		
	P1	P2	SNT	P1	P2	SNT
GABA	20.2	10.9	68.8	0.50	0.60	1.68
Aspartate	41.5	13.9	44.5	1.08	0.76	1.03
Glutamate	21.0	6.5	70.9	0.53	0.36	1.71
Glycine	23.8	11.2	64.9	0.58	0.62	1.57

Approximately 70 mg wet weight of rabbit retinae were homogenised in 20 volumes 0.32 M sucrose (6% w/v). Each retinal homogenate was spun at 1,000*g* for 5 min to produce a crude nuclear pellet (P1). The supernatant was then centrifuged at 17,000*g* for 30 min to produce a crude mitochondrial pellet (P2) and SNT (supernatant of latter centrifugation[12]). The amino acids in these fractions were extracted with ice-cold perchloric acid (6%) and allowed to stand overnight. The extracts were taken to *p*H 4.0 with KOH and centrifuged at 0° C to remove the perchlorate precipitate. The supernatant was acidified to *p*H 2.2 with citrate buffer and the amino acids were determined by ion-exchange chromatography using an automatic amino acid analyser (Biocal BC100). Results are expressed as both % of total recovered in P1 + P2 + SNT and relative specific activity (RSA = % total recovered in fraction/% total protein recovered in fraction). Results are means of 4–6 experiments. The s.e. of the means were between 0 and 10%.

but release slows in the light, resulting in hyperpolarisation of the horizontal cell[19,20]. Recently, Dowling and Ripps[21] have suggested that the photoreceptor transmitter may be aspartate or glutamate on the basis that increased Mg levels, which would be expected to reduce transmitter release, cause hyperpolarisation of second order retinal neurones. Since our experiments suggest that the P1 fraction contains most of the photoreceptor terminals, we have examined the distribution of endogenous aspartate, glutamate, GABA and glycine in the primary fractions produced from the rabbit retina (Table 1). These experiments indicate that the P1 fraction contains more than 40% of the aspartate, whilst only about 20% of the other amino acids are concentrated in this fraction. These results are consistent with the suggestion that aspartate may be the transmitter released from the photoreceptor terminals.

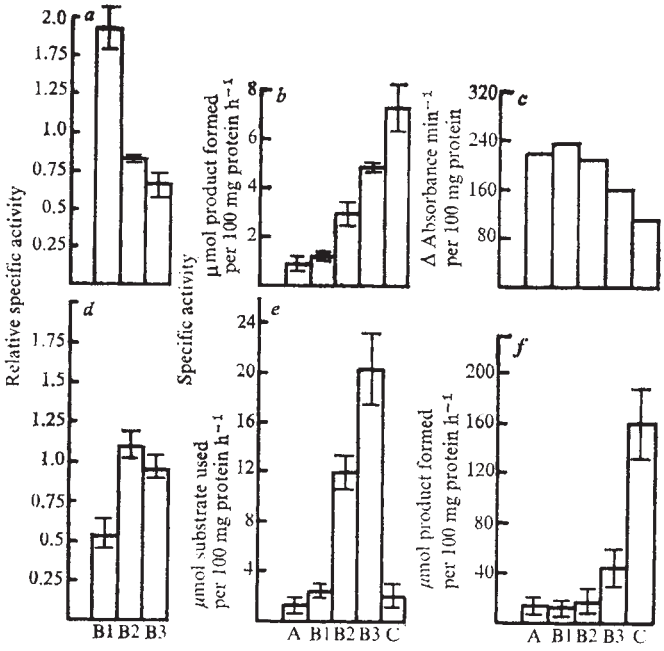

Fig. 2 Distribution of enzymes, ^{3}H-dopamine, and ^{14}C-GABA sucrose gradients. Retinae were incubated with a mixture of ^{3}H-dopamine (0. 1 μM) and ^{14}C-GABA (0.1 μM) for 20 min at 37° C in medium containing pargyline (15 μM), EDTA (25 μM), and ascorbic acid (1 mM). These retinae, and also unincubated retinae for enzyme assays, were homogenised in 0.32 M sucrose and centrifuged at 1,000*g* for 5 min. The supernatant was then spun at 17,000*g* for 30 min at 4° C to produce a crude mitochondrial pellet (P2). This pellet was resuspended in 0.32 M sucrose and layered on to the top of a four step discontinuous sucrose gradient (equal volumes of 1.4, 1.2, 1.0, 0.8 M sucrose) which was then centrifuged at 100,000*g* for 90 min at 4° C. The five resulting fractions were collected by aspiration. Fraction A being that floating on 0.8 M sucrose: C sedimenting below 1.2 M sucrose: The three intermediate fractions were designated B1, B2 and B3. The results are the means of 4 to 11 experiments, the vertical bars are the s.e. of the mean. *a*, ^{3}H-Dopamine; *b*, MAO; *c*, LDH; *d*, ^{14}C-GABA; *e*, GAD; *f*, GABA-T.

We have not yet been able to examine the morphology of the particles present in the various fractions obtained by density gradient centrifugation of the crude mitochondrial pellet (P2). The mitochondrial enzymes MAO and GABA-T, however, were predominantly localised in the pellet (C) (Fig. 2). The GAD activity was found predominantly in fraction B2 and B3. Since previous studies have shown that these fractions are rich in synaptosomes, and that GAD is concentrated in nerve endings[22-24], it seems likely that the retinal particles sedimenting in B2 and B3 represent 'gabainergic nerve endings'. Particles in these same fractions also accumulated most of the ^{14}C-GABA on the gradients; this coupled with previous electron microscope autoradiographical studies on cortical homogenates which have shown that labelled GABA is accumulated by nerve terminals[25], suggests that GABA-operated retinal nerve endings may also accumulate labelled GABA. But we cannot at present exclude the possibility that GABA-containing glial particles also sediment in these fractions.

^{3}H-Dopamine seemed to be localised preferentially in the B1 fraction suggesting that it was accumulated by slightly 'lighter' particles than those which accumulated ^{14}C-GABA. These particles are also likely to be synaptosomes since autoradiographic studies of ^{3}H-dopamine uptake in the retina have only revealed neuronal uptake[3,4].

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Newly synthesised lipids incorporated into influenza virus membranes

USING sequential labelling techniques, I have found that newly synthesised as well as preformed lipids are incorporated into the membrane of the maturing influenza virus, thus contradicting suggestions that only pre-existing

phospholipids[1-3] are utilised for the synthesis of enveloped viruses. This is not surprising in the light of reports that a selection mechanism exists for fatty acyl chains[4,5] (in ortho- and para-myxoviruses) and that many phospholipids[6] and neutral lipids[7] turn over rapidly in cultured animal cells.

Influenza virus can be considered a template virus—newly synthesised polypeptides are inserted into the membrane by lateral displacement of existing host cell polypeptides[8]. Studies of the origin of phospholipids have involved the use of ^{32}P-orthophosphate to measure the decay of phospholipids in the virion and infected cells. Although this technique appears to be valid for equilibrium labelling and thus for measuring the lipid composition of the virion[9], attempts to chase ^{32}P-orthophosphate from phospholipids in monolayer cultures have been unsuccessful. Since ^{32}P can exchange rapidly or compartmentalise into organic pools which turn over slowly, data for the turnover of phospholipids in chick embryo fibroblasts[7], obtained with this isotope would seem to be unreliable[8]. Using short pulses of 2-^{3}H-glycerol, Weinstein and I have already shown that the depression of synthesis of host cell lipids in virus-infected chick embryo fibroblasts depended on the multiplicity of infection, suggesting that newly synthesised lipids were incorporated into the virion[11].

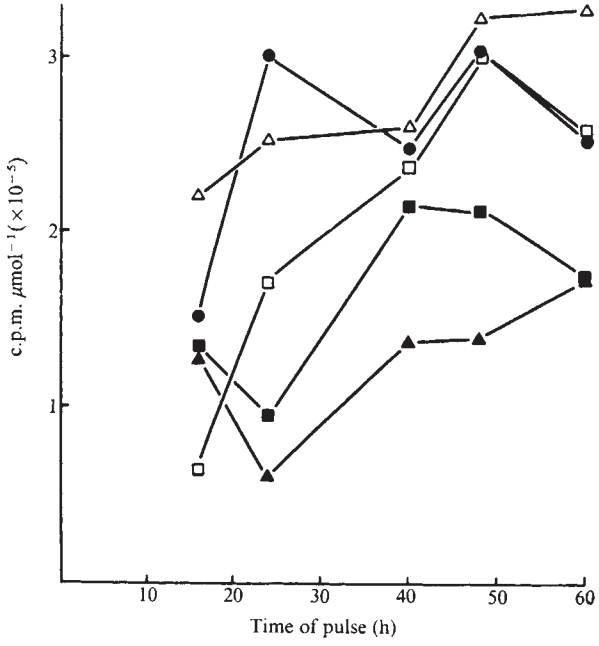

Fig. 1 Equilibrium labelling of chick embryo fibroblasts plated in the presence of 0.45 μCi ml^{-1} of 2-^{14}C-glycerol. △, Phosphatidylcholine; ■, phosphatidylethanolamine; ●, phosphatidylinositol; □, phosphatidylserine; ▲, sphingomyelin.

To investigate this question further, secondary chick embryo fibroblasts were seeded on to Falcon flasks (75 cm^2) in Puck's medium containing 10% calf serum and 0.45 μCi of 2-^{14}C-glycerol (ICN; specific activity 16 mCi mmol^{-1}). Individual flasks containing about 10^7 cells were sampled at various times, lipids were extracted and separated by thin-layer chromatography and their specific activity was determined[6]. With the exception of sphingomyelin, all phospholipids were labelled to equilibrium within 48 h (Fig. 1). To achieve maximal incorporation of label, confluent cells (at 48 h) were washed with KIR[6] and cells starved for 16 h in minimal Eagle's medium with 0.1% fatty-acid-poor bovine serum albumin (MEM-FAPBSA) containing 0.45 μCi ml^{-1} of 2-^{14}C-glycerol per ml. This step

Table 1 Incorporation of 2-^{3}H-glycerol into influenza virions derived from prelabelled cells

	Phospholipids c.p.m. μmol^{-1} Pi		
	^{14}C	^{3}H	^{3}H/^{14}C*
Lysophosphatidylcholine	14.8	10.4	0.7 (±0.1)
Phosphatidylcholine	315	312	1.0 (±0.1)
Phosphatidylserine	132	39	0.3 (NC)
Phosphatidylethanolamine	127	53	0.4 (±0.1)
Phosphatidylinositol	19	12	0.6 (±0.1)
Sphingomyelin	132	34	0.3 (NC)
	Neutral lipids c.p.m. μmol^{-1}		
	^{14}C	^{3}H	^{3}H/^{14}C*
Monoglycerides	208	83	0.4 (±0.1)
Free fatty acids	137	70	0.5 (±0.1)
Cholesterol	1964	200	0.1 (NC)
Diglycerides	172	241	1.4 (±0.15)
Triglycerides	91	227	2.5 (±0.2)
Cholesterol esters	250	83	0.3 (NC)

Doubly labelled virus was mixed with cold purified egg-propagated influenza virus, containing the equivalent of 300 μg of phospholipid, and purified by adsorption and elution from $BaSO_4$, two cycles of differential centrifugation and by banding (twice) in preformed gradients of potassium tartrate[12]. In a separate experiment, isotope dilution techniques[12] with ^{32}P revealed <0.1% contamination by host cell phospholipids as judged by Čerenkov radiation[13]. The extraction and quantification of labelled virus was as previously described[6,11]. Specific activities were determined after subtraction of the mean values obtained from duplicate runs of individual lipids obtained from cold purified virus from that of labelled virus plus an equivalent amount of cold virus which was separated by thin-layer chromatography. Corrections for background and overlap of channels was done directly in an Intertechnique spectrometer.
NC, no change: Pi, inorganic phosphate. Results represent means of three separate experiments.
*Bracketed figures represent variability of ratios between experiments.

further increased the incorporation of ^{14}C-glycerol by 50% (12–16 h were needed to achieve further equilibrium labelling, in the absence of serum). Monolayers were then washed with ice-cold KIR and infected with 15–20 plaque-forming units (PFU) per cell of influenza virus (Ao/WSN/34 strain) by adsorption at 4° C for 90 min. The virus inoculum was removed and the cells were pulsed with 1 μCi ml^{-1} of 2-^{3}H-glycerol (New England Nuclear Corp., specific activity 8.8 Ci mmol^{-1}) in MEM-FAPBSA for 16 h. Virus was collected after 16 h; the purification of virus and extraction and separation of lipids is described in the legend of Table 1.

Analysis of neutral glycerides showed that about 55–70% of di- and triglycerides were newly synthesised, whereas phosphatidylcholine contained half preformed and half newly synthesised lipid and in the case of phosphatidylethanolamine, about 30% was newly synthesised. Although glycerol labels cholesterol randomly, most of it appears to be synthesised before infection, confirming that this lipid turns over slowly.

These experiments provide direct evidence that newly synthesised lipids are incorporated into the virus (after infection). Since lipids are synthesised at the endoplasmic reticulum and transported to the cell surface membrane, it would appear that they must be transported to sites of viral membrane biogenesis which are 'hot spots'[14] for the integration of newly synthesised lipids as well as polypeptides coded for by the virus. The mechanism involved is unclear, but the most likely candidates for selective binding and transport of lipids to the sites of viral biogenesis are the envelope polypeptides and/or the 'M' protein[8]. These informational molecules determine the number and species of fatty acyl chains incorporated and provide a system for integration of lipids into membranes[8].

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Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria

WE have screened a large number of R plasmid-bearing *Escherichia coli* and obtained a few that confer resistance to the organomercurials phenylmercuric acetate (PMA) and methylmercuric chloride (MMA). Resistance to cationic Hg(II) in *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* has invariably been associated with plasmids that also mediate resistance to various antibiotics (R plasmids)[1-6]. The mechanism of mercury resistance is the enzymatic reduction of Hg(II) to Hg(0), which is volatile[2,7,8]. Previously, no organomercurial-reducing *E. coli* strains have been reported. The only organomercurial-reducing strain studied in detail is a PMA-resistant soil pseudomonad[7-9]. As organomercurials including PMA are a human health problem[10], it will be of interest to see if the mercury(ial) resistance of the bacteria in our alimentary canal influences the fate of ingested mercury(ials).

Starting with the Hammersmith Hospital collection[11,12] wherein over 800 enteric plasmids have been isolated and transferred into a common 'host' organism, an *E. coli* K-12 strain J53, each of the plasmid-bearing strains was tested by disk assay for resistance to both mercuric chloride (100 nmol per disk) and to phenylmercuric acetate (7 nmol per disk). A series of over 30 mercuric-resistant strains including some also resistant to PMA were tested for the ability to volatilise radioactive ^{203}Hg(II) and ^{203}Hg-PMA.

The distinction between sensitive and resistant strains is absolute with regard to volatilisation of Hg(II) and PMA (Fig. 1). Sensitive bacteria without plasmids such as strains J53-1 and AB1932-1 show no detectable loss of ^{203}Hg(II) or ^{203}Hg-PMA from the medium during 90 min incubation at 36° C. Strains harbouring R-factors that confer Hg(II)-resistance but not PMA-resistance volatilise only ^{203}Hg(II) and not ^{203}Hg-PMA (for example, strain J53(R222)); and strains that are resistant to both Hg(II) and PMA (such as strain J53(471a)) cause the volatilisation of both compounds.

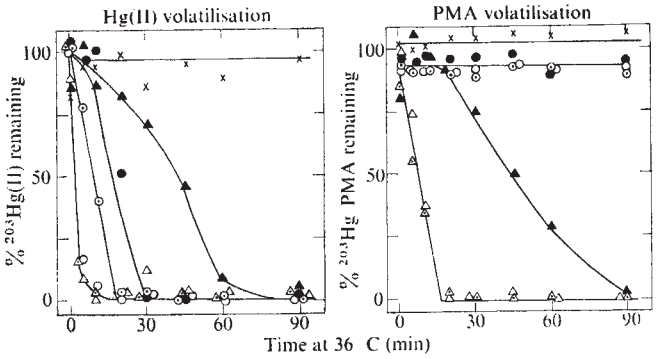

Fig. 1 Volatilisation of ^{203}Hg(II) and ^{203}Hg-PMA. Cells were grown overnight at 37° C in tryptone broth (8 g Difco Bacto-tryptone, 5 g NaCl l^{-1} deionised water) or in tryptone supplemented with 10 μM $HgCl_2$ or 1 μM PMA. In the morning, the cultures were diluted tenfold into the same medium and when growth reached 75 Klett units turbidity (number 54 filter; Klett-Summerson colorimeter), a third addition of Hg(II) or PMA was made to the induced cultures. After growth to 125 Klett units (230 μg dry weight ml^{-1}), the cells were collected by centrifugation at 6,000*g* for 10 min at 20° C and resuspended at 2.3 mg ml^{-1} (dry weight) in 0.01 M $NaPO_4$ buffer, *p*H 7.3. Cells were diluted elevenfold into an assay buffer containing final concentrations of 0.01 M $NaPO_4$ buffer, *p*H 7.3, 5×10^{-4} M Na_2EDTA, 10^{-3} M β-mercaptoethanol, 2×10^{-4}M $MgCl_2$ and either, *a*, 10 μM $^{203}Hg(NO_3)_2$ or *b*, 1.0 μM ^{203}Hg-PMA. 20 μl samples were removed periodically and counted by liquid scintillation spectroscopy[2]. The reaction mixtures (220 μl) were shaken at 200 r.p.m. in 10 × 75 mm test tubes in a 36° C water bath. ×, Uninduced J53-1; ●, uninduced J53(R222); ○, Hg(II)-induced J53(R222); ⊙, PMA-induced J53(R222); ▲ uninduced J53(471a); △ Hg(II)- induced J53(471a); ◬ PMA-induced J53(471a).

The results of a series of such experiments are summarised in Table 1 along with the antibiotic resistance pattern of each plasmid, the compatibility groups, and the bacterial species from which the plasmids were isolated. A small number of R factors from other sources and some hospital isolates were also tested. All of the 47 strains with mercury volatilising activity listed in Table 1 are inducible for this activity. This is seen in Fig. 1, where the initial rate of loss of ^{203}Hg(II) is five to ten times more rapid with bacteria that have been previously exposed to 10 μM $Hg(NO_3)_2$ or 1 μM PMA. With the strains harbouring the first eight plasmids that confer activity toward both Hg(II) and PMA, growth on either substrate induced the immediate rapid volatilisation of both—that is, a single system appears to be functioning or there is at least cross induction. With 38 of the remaining 39 Hg(II)-resistant strains, growth in the presence of 1 μM PMA induces the rapid volatilisation of ^{203}Hg(II), even though ^{203}Hg-PMA is not volatilised (that is, PMA appears to be a gratuitous inducer but not a substrate for the system).

As well as the two most common patterns (exemplified in Fig. 1 by plasmids R471a and R222), we have found a number of exceptional induction-volatilisation patterns, each of which is reproducible in repeated experiments with separate single colony isolates: First, strains with R factors R826 and R828 did not induce volatilisation of PMA in the assay conditions, although when pregrown with either Hg(II) or PMA, these strains rapidly volatilised PMA. Second, strains J53(R40a), RN180 and RN181 showed low level volatilisation of ^{203}Hg-PMA (±; < 50% of 1 μM PMA volatilised in 90 min at 36° C) as opposed to > 90% loss in 30 min with J54(R471a) (Fig. 1). Previous growth on 1 μM PMA induced rapid volatilisation of 10 μM Hg(II) by strains J53(R40a), RN180 and RN181, but little or no activity toward PMA. These strains belong to the class of

organomercurial-reducing strains, but they are quantitatively very different from the first eight plasmid-containing strains in Table 1. Third, with strain J53(R192) exposure to 1 μM PMA strongly inhibited (to less than one-fifth of the uninduced level) the ability of the cells to volatilise ^{203}Hg(II). Higher levels of PMA (approaching 10 μM) and $Hg(NO_3)_2$ (approaching 20 μM) have this effect on all the strains. Fourth, the strains carrying R factors R391 and R705 do not show induction of ^{203}Hg(II) volatilising activity during 90 min at 36° C in the buffer assay conditions.

We used phenylmercuric acetate for screening R factor-containing strains for resistance to organomercurials. Recently, we have measured the volatilisation of ^{203}Hg and ^{14}C from radioactive methylmercuric chloride when exposed to induced cells of J53(R471a), J53(R286) and J53-1(R831), but not when induced cells of these strains were used, nor with J53 derivatives with plasmids R15, R192, or R391 or the plasmidless J53-1 (all pre-exposed to Hg(II)). Strain RN180 induced with Hg(II) showed low level but significant volatilisation of ^{203}Hg-methylmercuric chloride.

Thirty-one of the mercury-resistant strains in Table 1 contain plasmids transferred into the common genetic background of K-12 derivative J53. Plasmids JJ1, U150 and U305 were transferred into another common K-12 derivative AB1932-1 (ref. 13) and have been previously shown to volatilise Hg(II)[2,4]. In terms of mercury resistance and volatilisation, AB1932-1(R15) is indistinguishable from J53-1(R15). The four RN plasmid-bearing mercury-resistant strains originated from Chabbert[14] in Paris. Two of

Table 1 Mercury and mercurial resistance R factors

Strain	Uninduced Hg(II)	Uninduced PMA	Hg(II) grown Volatilisation of: Hg(II)	Hg(II) grown Volatilisation of: PMA	PMA grown Hg(II)	PMA grown PMA	Plasmid characteristics: Antibiotic resistances	Compatibility group	Origin
J53-1	—	—	sensitive		sensitive		None		
J53 (R471a)	+	+	+	+	+	+	A, Cb, Cp, Tc	L	*Serratia marcescens* (Boston, Mass.)
J53 (R473)	+	+	+	+	+	+	A, Cb, Cp	L	*Serratia marcescens* (Boston, Mass.)
J53 (R826)	+	—	+	+	+	+	A, Cb, Cm, Gm, Km, Sm, Tc	S	*Serratia marcescens* (Toulon, France)
J53 (R828)	+	—	+	+	+	+	A, Cb Cm, Gm, Km, Sm, Tc	S	*Serratia marcescens* (Toulon, France)
J53-1 (R830a)	+	+	+	+	+	+	Km, Sm, Tc	L	*Serratia marcescens* (Boston, Mass.)
J53-1 (R831)	+	+	+	+	+	+	Km, Sm, Tc	L	*Serratia marcescens* (Boston, Mass.)
J53 (R832)	+	+	+	+	+	+	A, Cb, Km, Tc	L	*Serratia marcescens* (Boston, Mass.)
J53 (JR211)	+	+	+	+	+	+	A, Cb, Cm, Gm, Km, Sm, Su	A–C	*Klebsiella aerogenes*
J53-1 (R15)	+	—	+	—	+	—	Sm, Su, Sp, Su	N	Watanabe (Japan) (ref. 11)
J53 (R28)	+	—	+	—	—	+	Sm, Sp, Tc	FII	(ref. 11)
J53 (R40a)	+	—	+	±	+	—	A, Cb, Cp, Km, Su	A–C	*Pseudomonas aeruginosa*
J53 (R52)	+	—	+	—	+	—	Sm, Sp, Tc	FII	(ref. 11)
J53 (R57b)	+	—	+	—	+	—	A, Cb, Cm, Gm, Km, Su	A–C	*Klebsiella pneumoniae*
J53 (R192)	+	—	+	—	—	—	Cm, Tc	FII	(ref. 11)
J53 (R222)	+	—	+	—	+	—	Cm, Sm, Su, Tc	FII	Watanabe (Japan) (ref. 11)
J53-1 (R312)	+	—	+	—	+	—	Cm, Sm, Sp	FII	(ref. 11)
J53-1 (R348F)	+	—	+	—	+	—	Cm, Km, Sp, Tc	FII	(ref. 11)
J53 (R391)	—	—	+	—	+	—	Km, Tc	J	*Proteus rettgeri* (South Africa)
J53-1 (R401)	+	—	+	—	+	—	A, Cb, Sm	T	*Proteus rettgeri* (South Africa)
J53-1 (R455)	+	—	+	—	+	—	A, Cb, Cm, Sm, Su, Tc	FI	*Proteus morganii* (South Africa) (ref. 11)
J53 (R700)	+	—	+	—	+	—	Su	A–C	*Providencia* (Georgia, U.S.A.)
J53-1 (R702)	+	—	+	—	+	—	Km, Sm, Su, Tc	P	*Proteus mirabilis* (New Jersey)
J53-1 (R705)	—	—	+	—	+	—	Km, Tc	J	*Proteus vulgaris* (South Africa)
J53 (R718)	+	—	+	—	+	—	A, Cb, Cp, Km, Su, Tc	A–C	*Providencia* (Canada)
J53-1 (R725)	+	—	+	—	+	—	Cm, Sm, Su, Tc	O	*Shigella dysenteriae* (Mexico)
J53 (R754)	+	—	+	—	+	—	A, Cb, Cm, Cp, Sm, Su		*Proteus mirabilis* (Thailand)
J53 (R938)	+	—	+	—	+	—	A, Cb, Cm, Cp, Km, Sm, Tc, Su	A–C	*Serratia marcescens*
J53-1 (JR72)	+	—	+	—	+	—	Cb, Cm, Km, Sm, Sp, Tc	FII	Julian Davies (Wisconsin)
J53-1 (RH28)	+	—	+	—	+	—	A, Cb, Cm, Cp, Tc	S	*Serratia marcescens*
J53-1 (RIP69)	+	—	+	—	+	—	A, Cb, Cp, Km, Tc	M	Chabbert (Inst. Pasteur, Paris) (ref. 16)
J53 (RIP135)	+	—	+	—	+	—	A, Cb, Cp, Gm, Sm, Su, Tc	M	Chabbert (Inst. Pasteur, Paris) (ref. 16)
AB1932-1	—	—	sensitive		sensitive		None		(refs 2 and 13)
AB1932-1 (JJ1)	+	—	+	—		—	Cm, Sm, Sp, Su	FII	*E. coli* (ref. 13)
AB1932-1 (R15)	+	—	+	—	+	—	Sm	N	Introduced by R. Hedges
AB1932-1 (U150)	+	—	+	—	+	—	A, Sm, Su, Tc		(ref. 4)
AB1932-1 (U305)	+	—	+	—	+	—	Cm, Sm, Tc		(ref. 4)
CR34 (R100-1)	+	—	+	—	+	—	Cm, Sm, Tc		
RN180	+	—	+	±	+	±	A, Cb, Km, Su	A–C	*Providencia;* Chabbert No. 13–84
RN181	+	—	+	±	+	±	A, Cb, Cm, Cp, Km, Sm, Su, Tc	A–C	*Providencia;* Chabbert No. 13–84
RN182	+	—	+	—	+	—	Cm, Sm, Su		*Salmonella paratyphi B.* (ref. 14)
RN184	+	—	+	—	+	—	A, Cb, Cm, Cp, Sm, Su		*Salmonella;* ref. 17
DG21	+	—	+	—	+	—	Cp, Sm, Tc		*E. coli*, Rochester, New York
DG35	+	—	+	—	+	—	A, Cb, Cm, Km, Sm, Tc		David Groves (ref. 15)
DG36	+	—	+	—	+	—	A, Cb, Cm, Km, Sm, Tc		
B36	+	—	+	—	+	—	A, Cb, Cm, Sm, Tc		
J71	+	—	+	—	+	—	A, Cb, Cm, Km, Sm, Tc		*E. coli*, St Louis, Missouri
J82	+	—	+	—	+	—	A, Cb, Cp, Tc		
BU9990	+	—	+	—	+	—	Sm, Tc		

The plasmids in strains J53 or J53-1 (a derivative of J53 with a chromosomal mutation to nalidixic acid-resistance) come from the Hammersmith Hospital collection[11,12]. R factors R15, R28, R52, R192, R222, R312 and R348F have been previously reported[11] as mercury resistant. Plasmid RIP69 has been described[16] and was assigned to compatibility class 7, later renamed group M (ref. 12). Compatibility groups 'L' and 'S' will be described shortly[18]. Plasmid R15 was introduced by conjugation into an acridine-orange 'cured' plasmid-less derivative of AB1932-1 (J31); Su resistance was not tested after transfer. Plasmid R100 is a synonym for plasmid R222 [17]; Su resistance and compatibility group were not tested with CR34 (R100-1). The symbols for antibiotic resistances are: A, ampicillin; Cb, carbenacillin; Cp, cephalothin; Cm, chloramphenicol; Gm, gentamycin; Km, kanamycin; Sm, streptomycin; Sp, spectinomycin; Su, sulphonamide; Tc, tetracycline. The antibiotic resistances are those tested in this study and therefore incomplete; the resistance to the penicillin class of antibiotics does not necessitate several enzymic mechanisms.

these seem identical to our most common class of plasmids conferring only mercury resistance, represented in Fig. 1 by R222. The other two, RN180 and RN181, represent an exceptional class. A series of hospital isolates from Rochester, New York (DG21, DG35 and DG36)[13] and from St Louis (J71, J82, B36 and BU9990) complete our current collection of mercury volatilising *E. coli*. In these strains, the plasmid nature of the mercury resistance has yet to be shown but all are multiply antibiotic resistant. The frequency of mercury resistance amongst the new St Louis isolates was 4/126 (4 of the 25 new antibiotic resistant isolates). Approximately 200 of the 800 plasmids from various enteric organisms that have been transferred into J53 confer resistance to Hg(II). The first seven of the plasmids conferring resistance to PMA and Hg(II) originated in *Serratia marcescens* isolates. Five belonged to the 'L' incompatibility group and came from Boston, Massachusetts; the other two were group 'S' and came from Toulon, France. Recently, however, strain J53 containing plasmid JR211, which belongs to the 'A-C' compatibility group and originated in *Klebsiella aerogenes*, was found to volatilise ^{203}Hg-PMA. Clearly, the frequency of mercury resistance is sufficiently high to make this a useful epidemiological marker and further studies with mercury and mercurial resistance patterns should help in elucidating relationships between plasmids.

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Unidirectional replication of plasmid ColE1 DNA

COLICINOGENIC plasmid E1 (ColE1) ha been isolated from *Escherichia coli* both as a supercoiled DNA molecule of 4.2×10^6 molecular weight and as a supercoiled DNA-protein relaxation complex[1]. Treatment with certain agents that denature protein structure induces conversion of the supercoiled ColE1 DNA-protein complex to an open circular (relaxed) DNA form with one single strand break specifically in the poly (U,G) binding or 'Crick' strand[2,3]. It has been shown that the EcoR1 restriction endonuclease cleaves ColE1 DNA at a single site. Using that site as a reference point it was demonstrated that the single strand break in the open circular product of induced relaxation is located at a unique site, approximately 19% of the length of ColE1 DNA from one end of the EcoR1 cleaved molecule[4]. Only certain restriction endonucleases have previously been shown to cleave DNA at a specific nucleotide sequence and the result of their action is a double strand break[5–7].

Replicating ColE1 DNA molecules have been isolated from *E. coli* cells and examined with respect to their physicochemical properties (L. K. Williams S. Sato, R. W. Leavitt, and D.R.H., manuscript in preparation). The parental strands of these ColE1 molecules, like those of replicating SV40 molecules[8,9], are covalently closed as determined by their broad distribution between the densities of supercoiled and open circular DNA in caesium chloride–ethidium bromide density gradients, by their rapid sedimentation in alkaline sucrose density gradients and by their appearance in the electron microscope as partially supercoiled, partially open structures. Fareed *et al.*[10] used the single EcoR1 cleavage site in SV40 DNA as a reference point to determine that SV40 DNA replication proceeds bidirectionally from a unique origin. We describe here an analysis of EcoR1 cleaved replicating ColE1 DNA molecules. Evidence is presented that ColE1 DNA replication proceeds unidirectionally from an origin whose location is indistinguishable from the site of the single-strand break present in the open circular DNA of the relaxed ColE1 complex.

A lysate of *E. coli* JC411 cells, colicinogenic for the plasmid ColE1, prelabelled with ^{14}C-thymine and pulse labelled with ^{3}H-thymidine was centrifuged to equilibrium in a caesium chloride–ethidium bromide density gradient. The pulse labelled DNA was separated into two pools, one comprising ^{3}H-DNA of a density intermediate between that of supercoiled ColE1 DNA and open circular or linear DNA and the second comprising ^{3}H-DNA of a density equal to that of open circular or linear DNA. ColE1 replicating intermediates in the less dense pool consist of molecules that have replicated to a greater extent than the replicative forms in the intermediate density pool of DNA (L. K., P. H. Williams, S. Sato, R. W. Leavitt and D.R.H., manuscript in preparation). Similar observations have been made for replicating SV40 molecules[8,9]. To purify the ColE1 DNA further, each pool was centrifuged on preparative sucrose density gradients and the ^{3}H-DNA which sedimented as rapidly or more rapidly than 23S DNA (the sedimentation coefficient of supercoiled ColE1 DNA) from each preparative gradient was pooled separately.

A sample of each of the two pools was treated with the EcoR1 endonuclease and analysed by centrifugation through 5–20% sucrose density gradients. Figure 1*a* shows that before cleavage the ^{3}H-thymidine-labelled pool of DNA originally derived from the intermediate density peak in the caesium chloride–ethidium bromide density gradient sedimented with coefficients of 23–34S. There is a peak of the prelabelled ^{14}C-thymine-labelled DNA at the 23S position, presumably arising by contamination from the supercoiled peak in the preparative dye–caesium chloride density gradient. After cleavage (Fig. 1*b*) much of the ^{14}C-DNA was found in the 15S position expected for the linear ColE1 DNA. The pulse

labelled ^{3}H-DNA sedimented with sedimentation coefficients between 15S and 23S after cleavage. Figure 1*c* shows that before cleavage the pool of ^{3}H-DNA derived initially for the lowest density peak in the caesium chloride–ethidium bromide density gradient sedimented rapidly between 23S and 42S with most of the ^{3}H-DNA counts between 38S and 42S. Again much of the prelabelled ^{14}C-DNA sedimented in the position of 23S supercoiled ColE1 DNA. After cleavage with the EcoR1 endonuclease (Fig. 1*d*) most of the ^{14}C-DNA sedimented at 15S while most of the ^{3}H-DNA sedimented with coefficients between 17S and 23S. The rapid sedimentation through neutral sucrose density gradients, characteristic of replicating ColE1 DNA before and after cleavage, is consistent with the observations in the electron microscope that before cleavage with EcoR1, replicating ColE1 molecules exhibit a partially supercoiled, partially open structure and that after cleavage with EcoR1 linear molecules are observed containing an internal loop of replicated DNA and two unreplicated branches. Figure 2 represents typical EcoR1-cleaved ColE1 DNA molecules in various stages of replication.

The total lengths of the linear molecules with internal loops derived from EcoR1-cleaved DNA of the intermediate and light density pools, were determined by electron microscopy. The linear molecules with internal loops were found to be of the same length as the contour length of open circular ColE1 DNA spread on the same grid to a 95% confidence level. An analysis of the changes in relative lengths of the unreplicated branches as the extent of replication changes is shown in Fig. 3 for 124 ColE1 DNA molecules that were between 3 and 70% replicated. It is clear that as replication proceeds, one unreplicated arm of the molecule, designated U_1, remains constant in size and the other unreplicated arm, U_2, progressively decreases in size. This is as expected for a unidirectional mode of replication from a fixed origin. In contrast, if replication proceeded bidirectionally from a fixed origin, both U_1 and U_2 would decrease in size with an increasing extent of replication. Extrapolation of U_1 and U_2 to 0% replication permits a determination of the position of the origin of ColE1 DNA replication. As Fig. 3 shows, the origin is calculated to be 18.3±0.9% of unit length from one EcoR1 end and 81.7% from the other.

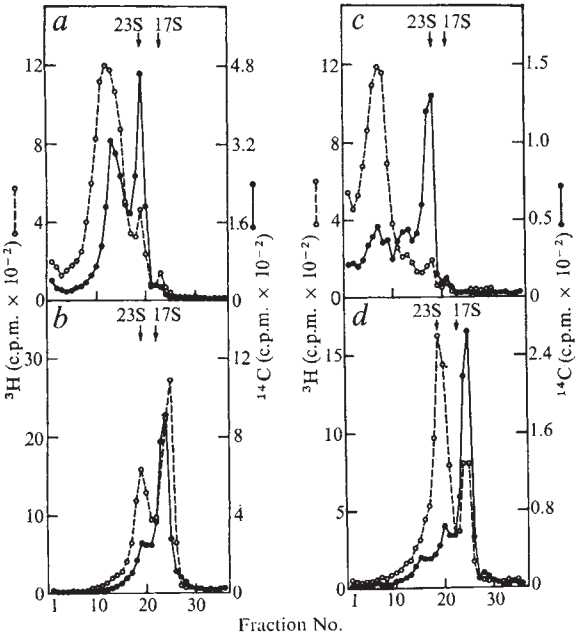

Fig. 1 Neutral sucrose sedimentation analysis of replicating ColE1 DNA molecules untreated and treated with the EcoR1 restriction endonuclease. A 500 ml culture was prelabelled uniformly with ^{14}C-thymine (0.1 μCi ml^{-1}) to a density of 5×10^8 cells ml^{-1}, collected and resuspended in medium lacking thymine and containing 3 mM cyclic AMP. After 30 min, the cells were pulsed for 30 s with ^{3}H-thymidine (10 μCi ml^{-1}) and 0.3 μg ml^{-1} of unlabelled thymidine. The cells were collected by centrifugation and lysed by the SDS-salt method[11]. The pulse label was purified by neutral sucrose gradient sedimentation and caesium chloride–ethidium bromide density gradient centrifugation (L.K., Williams, Sato, Leavitt and D.R.H., manuscript in preparation). Two peaks from the dye-buoyant density gradient containing replicating ColE1 DNA molecules, one banding at the intermediate density (*a* and *b*), the other at the light density (*c* and *d*) were sedimented through neutral 5–20% sucrose density gradients before (*a* and *c*) and after (*b* and *d*) treatment with the EcoR1 restriction endonuclease. Approximately 1 μl of an EcoR1 enzyme preparation was used for each 100 μg of ColE1 DNA in 0.1 M Tris, *p*H 7.5, 50 mM NaCl and 10 mM $MgCl_2$. After 5 min at 37° C the reaction was stopped by the addition of EDTA (final concentration of 25 mM).

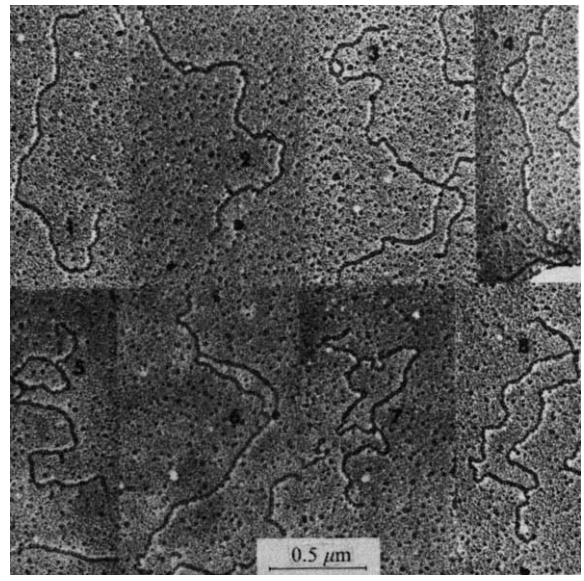

Fig. 2 Replicating ColE1 DNA molecules treated with the EcoR1 restriction endonuclease. DNA was spread by the aqueous technique of Davis *et al.*[12], rotary shadowed with platinum–palladium, and examined in the Phillips 200 electron microscope. Micrographs of selected molecules are arranged in order of increasing extent of replication. Panel 1 represents an unreplicated linear molecule.

Since replication proceeds unidirectionally, molecules greater than 81.7% replicated would be expected to have a double-Y appearance after cleavage with EcoR1. Several double Y molecules have been found in the ^{3}H-DNA derived from the light density peak in the preparative dye–caesium chloride density gradient and representative molecules are shown in Fig. 4. One of the forks of these molecules has two branches of equal size of approximately 82% of the total length of ColE1 DNA joined by a short segment to a fork with two smaller branches of equal size. As the size of the two smaller branches increases, the length of the short segment decreases. The structure of these double Y molecules is consistent with a unidirectional mode of replication from a fixed origin.

A consequence of unidirectional replication of a circular DNA molecule from a unique origin is that the origin and the termination point for replication are juxtaposed. The position of the origin-terminus for ColE1 DNA replication, 18.3±0.9% from one end of the EcoR1 cleaved molecule, is indistinguishable from the location of the single strand

break present in the open circular DNA of the relaxed ColE1 DNA-protein complex (approximately 19% of unit length from one end of the EcoR1-cleaved molecule). While these data do not distinguish between the two ends of the EcoR1-treated molecule, the apparent correspondence of the nicked site and the origin-terminus implies a direct involvement of the protein components of the relaxation complex in ColE1 DNA replication.

Other plasmids studied in *E. coli*, including several different sex factors, Col, and R plasmids, have also been isolated in the form of DNA-protein relaxation complexes[13]. The relaxation event has been shown to be strand specific for ColE2 (ref. 3), F1 (ref. 14) and R6K (Y. Kupersztoch, M.A.L. and D.R.H., submitted for publication) as well as for ColE1, and it has been demonstrated that the single strand break found in the open circular DNA after induced

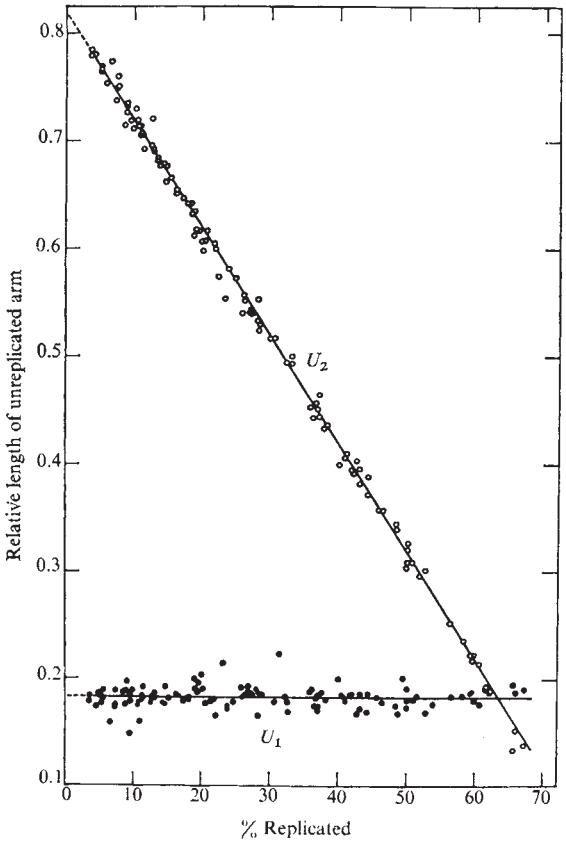

Fig. 3 Analysis of the length of the two unreplicated segments of replicating ColE1 DNA molecules after cleavage with EcoR1 as a function of the extent of replication. Replicating ColE1 DNA molecules purified and treated with the EcoR1 restriction endonuclease as described in Fig. 1 were used in this analysis. U_1 and U_2 are the unreplicated branches joining an internal loop comprised of R1 and R2. The relative lengths of U_1 and U_2 for each molecule was determined from the formula $T = U_1 + U_2 + 1/2(R_1 + R_2)$, where T = total length. The percentage replicated $= 1/2 (R_1 + R_2)/T$.

relaxation of the ColE2 and PSC101 (ref. 4) and R6K (Y. Kupersztoch, M.A.L. and D.R.H., manuscript submitted for publication) complexes is located at a unique site in each case. The replicon hypothesis of Jacob *et al.*[15] advanced the idea that DNA replication is regulated by the action of a protein, the initiator, at the origin of replication, the replicator locus. It is possible that these specific complexes of plasmid DNA and protein reflect the basic organisation of plasmids in *E. coli* as autonomous units of replication.

Since the strand specific break found as a result of induced relaxation is at the origin-terminus of ColE1 DNA replication, it is conceivable that the protein components of the

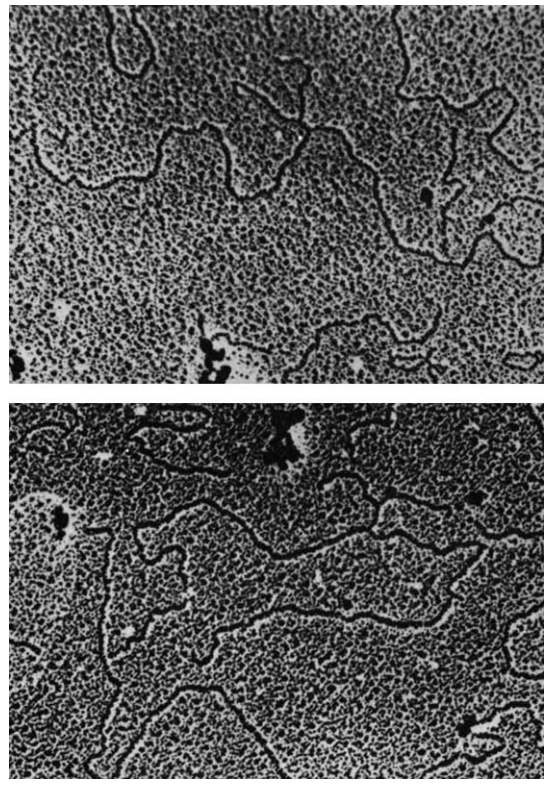

Fig. 4 Double Y structures derived from EcoR1 treatment of replicating ColE1 DNA molecules. Replicating ColE1 DNA molecules were purified and treated with EcoR1 as described in Fig. 1.

relaxation complex could be involved in the initiation of DNA synthesis and/or in its termination. At least in the case of the ColE1 DNA-protein relaxation complex it has been shown that the protein components of the complex have novel properties that could be consistent with a direct role in DNA synthesis. The supercoiled ColE1 complex has three major protein components, whose molecular weights are approximately 60,000, 16,000 and 11,000 (M.A.L. and D.R.H., manuscript in preparation). After the induction of relaxation by treatment with sodium dodecyl sulphate (SDS), the 16,000 and 11,000 molecular weight proteins are completely released from the open circular DNA and the 60,000 molecular weight protein is found in an association, proposed to be covalent, with the broken strand (M.A.L. and D.R.H., manuscript in preparation) at its 5′ terminus (D. G. Guiney and D.R.H., manuscript in preparation). Such nicking and covalent joining *in vivo*, augmented by a capability to reseal the nicked DNA, could provide the swivel mechanism thought to be necessary to facilitate the unwinding of the parental strands of replicating DNA. Activity as a swivel mechanism might be required both before and after the initiation of DNA synthesis. A nicking and resealing mechanism could also be active at the termination point of DNA synthesis to facilitate the separation of two completed but interlocked molecules.

It remains to be determined whether or not unidirectional replication is an obligatory feature of the plasmid state (perhaps reflecting an essential role for relaxation complex in initiation and termination of DNA synthesis). The identification of a plasmid with the property of bidirectional DNA synthesis coupled with knowledge of the location of the site nicked by the relaxation complex could resolve the question of the action of the relaxation complex at either the origin or at the terminus of DNA replication.

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Rifampicin-resistant replication of coliphage N4 DNA

IT has recently been reported that rifampicin-sensitive RNA synthesis participates in the replication of bacterial[1,2], episome or plasmid[3-8] and phage[7-11] DNA, presumably in a primer role during initiation. A more complex model of DNA replication may, however, be provided by the development of coliphage N4 (ref. 12). Transcription of N4 chromosome is unusual in that the virus uses two new rifampicin-resistant polymerising activities[13]. Expression of the early and delayed-early genes is therefore unaffected in rifampicin-pretreated cells although infection is abortive since transcription of the late N4 genes requires the functioning of the rifampicin-sensitive *Escherichia coli* RNA-polymerase (ref. 14). Here we show that initiation and continuation of N4 DNA replication take place in conditions in which the host RNA polymerase is totally inactive and give an account of the nature of the products that are synthesised in rifampicin-pretreated cells.

As shown in Fig. 1, rifampicin (200 μg ml^{-1}) added to Hfr 3300 cells 10 min before infection, did not prevent host DNA shut-off and subsequent phage DNA replication. N4 DNA synthesis was always initiated with a 3–5 min delay and the rate of ^{3}H-thymidine incorporation reached from 50–60% of that observed during normal infection. The bulk of the newly produced DNA is viral, since all of the DNA-negative *amber* mutants[15] tested failed to induce DNA synthesis. The effect on phage-coded macromolecular syntheses of adding rifampicin at different times before infection suggests that the reduction in the rate of DNA synthesis is caused by the decreased protein synthesis rather than by a direct effect of the drug on DNA replication (Fig. 2). As practically no incorporation of ^{3}H-uridine into RNA was observed in uninfected cells pretreated for 10 min with rifampicin it can be concluded that N4-directed DNA production can occur in the total absence of transcription by the host RNA-polymerase. It should be stressed, however, that N4-infected rifampicin-pretreated cells do synthesise large amounts of RNA (Fig. 2). Rothman-Denes and Schito have demonstrated that this rifampicin-resistant RNA hybridises specifically only to N4 DNA (ref. 13).

The nature of the DNA synthesised in the presence of rifampicin was subsequently investigated. Earlier studies showed that N4 parental DNA rapidly attaches to the cell membrane and that most of the ^{3}H-thymidine incorporated in a 1 min pulse administered at any time after the onset of phage DNA synthesis is complexed to fast sedimenting bacterial sites[16] where replication takes place (intermediate I)[17]. Maturation of the replicated DNA to the 40S molecule contained in the virion[18] requires the expression of late phage functions. Infection performed in rifampicin-pretreated cells resulted in the association of approximately 85% of the ^{32}P-labelled parental N4 chromosome with the cell membrane (not shown) implying that RNA synthesis mediated by the host RNA polymerase is not required for attachment. The sedimentation profile of the pulse-labelled DNA synthesised in the presence of the drug is furthermore undistinguishable from that observed under normal conditions, indicating that rifampicin does not prevent the parental genome from replicating in association with the correct bacterial site (Fig. 3*a* and *b*). When the fate of the

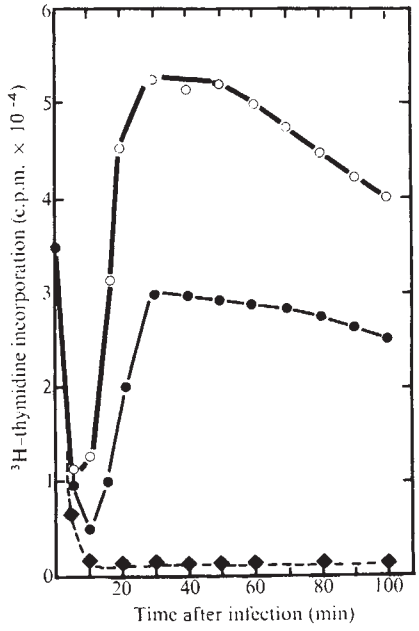

Fig. 1 Effect of rifampicin on N4 DNA synthesis. *E. coli* K12 strain Hfr 3300 was grown at 37° C in M9S casamino-acids-glucose medium[12] to a density of about 2×10^8 cells ml^{-1}. The culture was divided into several portions of equal volume (10 ml). One portion served as a control while 200 μg ml^{-1} of rifampicin (Lepetit) was added to the other portions. After shaking at 37° C for 10 min, wild type or *amber* 8, 12, 15, 23 or 25 DNA-negative (DO) N4 mutants were added separately at a multiplicity of infection of 10. At specified times thereafter 0.2 ml aliquots were removed and incubated for 2 min with methyl-^{3}H-thymidine (10 μCi ml^{-1}, 14 Ci mmol^{-1}, Amersham, England). At the end of the pulse 0.05 ml samples were precipitated with trichloroacetic acid and counted in toluene PPO as previously detailed[15]. O, N4 wild-type-infected culture, untreated; ●, N4 wild-type infected culture, pretreated with rifampicin; ♦ DO-*amber* mutant-infected cultures, pretreated with rifampicin.

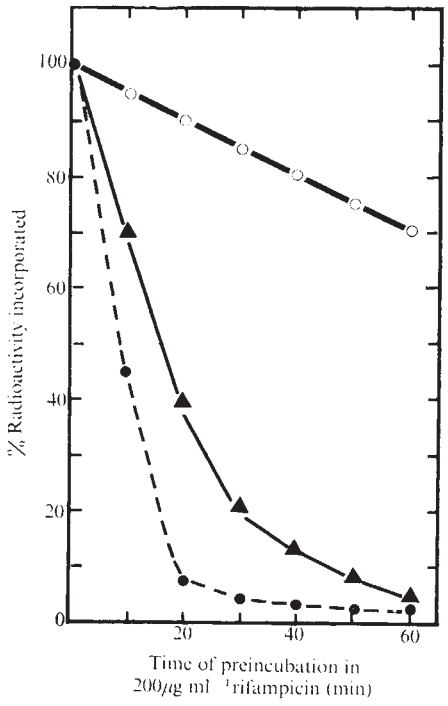

Fig. 2 Rate of macromolecular syntheses in Hfr 3300 cells incubated with rifampicin for different lengths of time and infected with coliphage N4. Cells were grown according to the procedure described in the legend of Fig. 1. Rifampicin (200 μg ml^{-1}) was added to different cultures 0, 10, 20, 30, 40, 50 and 60 min before infection with N4 at a multiplicity of 10. To measure incorporation of labelled precursor into DNA, 0.2 ml samples were removed 20 min after the addition of the phage and pulsed for 2 min with methyl-^{3}H-thymidine (10 μCi ml^{-1}). RNA synthesis was assessed by pulsing 0.2 ml aliquots from 6–8 min postinfection with ^{3}H-uridine (5 μCi ml^{-1}, 41 Ci mmol^{-1}; Amersham) and protein synthesis was determined by labelling for 3 min 0.2 ml portions, taken 5 min after infection, with ^{3}H-leucine (20 μCi ml^{-1}, 0.125 Ci mmol^{-1}; Amersham). The TCA-insoluble radioactivity incorporated was measured as described[15]. Values are plotted as a percentage of the radioactivity incorporated by Hfr 3300 cells treated with rifampicin at time zero of infection. On the ordinate 100 corresponds to 41,400 c.p.m. for ^{3}H-thymidine; 67,000 c.p.m. for ^{3}H-uridine and 8,300 c.p.m. for ^{3}H-leucine. ▲, Isotope incorporation into DNA; ○, into RNA; ●, into protein.

DNA contained in intermediate I was analysed by chasing the radioactivity incorporated during the pulse with an excess of unlabelled thymidine, strikingly different results were observed depending on whether intermediate I was formed in the presence of rifampicin or not. As shown in Fig. 3*c* in normal conditions most of the pulse-labelled DNA in intermediate I was released as material sedimenting with a rate characteristic of mature N4 DNA after a chase of 20 min, whereas none of the DNA species synthesised in drug-pretreated cells could be chased into slowly sedimenting material (Fig. 3*d*). Analysis by neutral or alkaline sucrose density gradient centrifugation of the DNA accumulated during continuous labelling of infected cells confirmed that upon expression of late phage functions the newly replicated DNA gives rise to molecules possessing the same hydrodynamic properties shown by N4 mature polynucleotide chains (Fig. 4*a* and *c*). Detachment and maturation of N4 DNA are possibly interdependent events since in rifampicin-pretreated cells not only did detachment from the bacterial site fail to occur (Fig. 4*b*) but the polynucleotide chains accumulated in intermediate I, upon denaturation by alkali migrated as an heterogeneous peak with much of the material sedimenting faster than single strands of mature DNA (Fig. 4*d*). The lack of transcription, in the presence of the antibiotic, of the late phage genes[13] which code for maturing factors could explain the accumulation of the fast sedimenting DNA species.

The extended synthesis of viral DNA in cells pretreated with rifampicin (Fig. 1) indicates that the host RNA-polymerase is not required in the initiation and elongation reactions of N4 chromosome replication. These findings are in contrast with those obtained with other host–phage systems in which rifampicin-sensitive transcription has been shown to be a prerequisite for at least the initiation of replication[7–11]. One might therefore assume either that RNA synthesis in general is dispensable for N4 DNA replication or that RNA priming is required for initiation but is carried out by a system distinguishable from the host RNA-

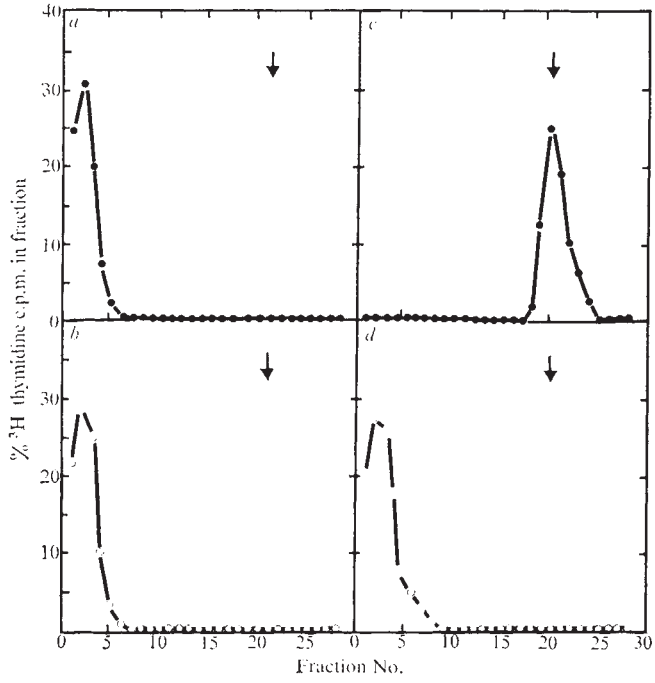

Fig. 3 Sedimentation analysis of N4 DNA synthesised in control cells and in cells pretreated with rifampicin. *E. coli* Hfr 3300 was grown at 37° C in M9S with shaking. When the cell density reached 2.5×10^8 cells ml^{-1} the culture was divided into two portions. One portion was left untreated and served as a control and 200 μg ml^{-1} of rifampicin was added to the other aliquot. After 10 min incubation at 37° C N4 was added at a multiplicity of 10. Newly synthesised phage DNA was labelled from 20–22 min after infection by the addition of methyl-^{3}H-thymidine (14 Ci mmol^{-1}) to a final concentration of 20 μCi ml^{-1}. At the end of the labelling period a 2 ml sample was taken and processed as described below. To the remaining culture unlabelled thymidine was added to a final concentration of 2.0 mg ml^{-1}. At 40 min after infection a sample of cells was centrifuged and gently lysed by the Tris-lysozyme-EDTA-detergent method described by Hallick Boyce and Echols[21]. Portions of the cell lysates (0.15 ml) were mixed with ^{32}P-N4 DNA (ref. 17) and centrifuged in a 4.2 ml of 5–20% sucrose gradient overlaying a 0.3 ml 1.7 g ml^{-1} caesium chloride shelf. Sucrose solutions were made up in 0.02 M Tris-HCl, *p*H 8.0, containing 0.01 M EDTA. Centrifugation was in the Spinco SW50 rotor for 60 min at 35,000 r.p.m. and 5° C. Fractions (about 0.15 ml) were collected from the bottom of the tube and the acid-insoluble radioactivity in a 0.05 ml sample was determined. Overall recoveries of ^{3}H were greater than 85%. Labelled N4 DNA found at the CsCl-sucrose underlayer is defined as Intermediate I. The arrows indicate the position to which marker ^{32}P-N4 DNA sediments. *a*, Sedimentation pattern of pulse-labelled N4 DNA; control cells; *b*, sedimentation pattern of pulse-labelled N4 DNA: cells pretreated with rifampicin; *c*, fate of pulse-labelled N4 DNA after a 20 min chase with unlabelled thymidine: untreated cells: *d*, fate of pulse-labelled N4 DNA: cells pretreated with rifampicin.

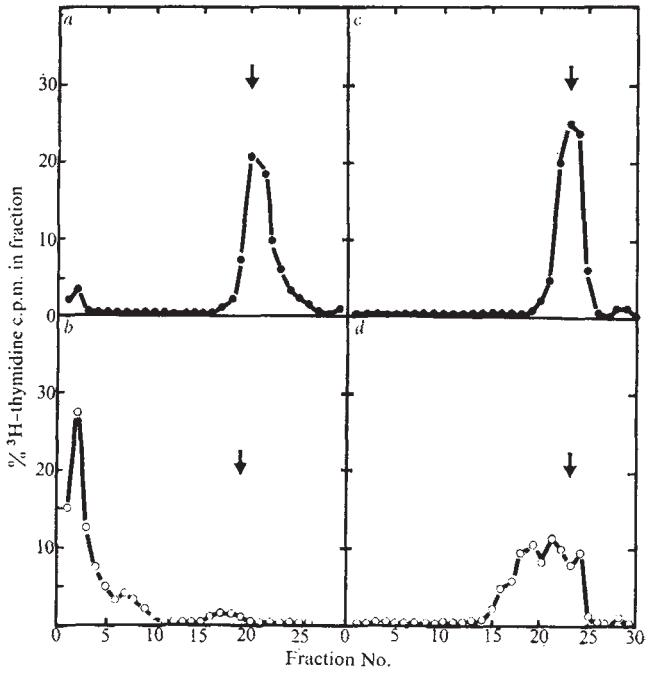

Fig. 4 Sedimentation analysis of N4 DNA labelled continuously in control cells and in cells pretreated with rifampicin. Hfr 3300 was grown as described in the legend of Fig. 1. At a cell density of 2×10^8 cells ml^{-1} the culture was divided into two aliquots. One portion served as a control while 200 μg ml^{-1} of rifampicin was added to the other portion. After 10 min incubation at 37° C N4 was added at a multiplicity of 10. Phage DNA was continuously labelled from 20 to 40 min after infection by the addition of methyl-^{3}H-thymidine (14 Ci $mmol^{-1}$) to a final concen tration of 5 μCi ml^{-1}. At the end of the labelling period, samples were taken and DNA was gently extracted as specified in the legend of Fig. 3. Portions of the cell lysate (0.15 ml) were mixed with ^{32}P-N4 DNA and centrifuged in neutral or alkaline sucrose gradients. Alkaline sucrose solutions were made up in 0.001 M EDTA and 0.3 M NaOH. Conditions of sedimentation and analysis were the same as those described in Fig. 3. *a* and *c*, Patterns of sedimentation of N4 DNA labelled continuously from 20–40 min after infection in control cells. *a*, Neutral sucrose gradient; *c*, alkaline sucrose gradient. Patterns of sedimentation of N4 DNA labelled continuously for the same period of time in rifampicin-pretreated cells. *b*, neutral sucrose gradient; *d*, alkaline sucrose gradient. The position to which marker ^{32}P-N4 DNA sediments is indicated by the arrows.

polymerase. The rifampicin-resistant mode of initiating Okazaki pieces[19], the replicative form of phage ΦX 174 (ref. 20) and the λ*dv* plasmid dimer[9] could, in principle, be used by N4 DNA for priming the next round of replication. More likely, one or both of the phage-related RNA-polymerising activities[13] may be involved in a drug-resistant transcription both at the origin and during elongation of N4 DNA. Finally, an altogether different mechanism of DNA strand initiation and elongation cannot be ruled out on the basis of present data. Isolation of a ribopolynucleotide covalently attached to a growing viral deoxypolynucleotide chain may place the mode of N4 DNA replication in a more usual frame and may render this system amenable to further investigation.

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Poly(C) in animal viral RNAs

A TRACT of approximately 100 nucleotides, containing 80–90 cytidylic acid residues (poly(C)) has been found within the genome of encephalomyocarditis virus[1] (EMCV). We have sought to establish whether this unusual structural feature is also present in other animal picornaviruses, including those of human and veterinary importance, such as rhino-, foot-and-mouth disease and swine vesicular disease viruses. The composition and extent of occurrence of poly(C) in a variety of picornaviruses might indicate whether it has a role in any of the. processes which are common to all of these viruses, such as penetration, replication and assembly[2]. Furthermore, if poly(C) is a virus-specific feature, it might prove feasible to devise antiviral chemotherapeutic strategies based upon its presence.

Picornaviruses (family Picornaviridae) have been classified[3,4] into five subgroups on the basis of physicochemical evidence; namely the enteroviruses, cardioviruses, foot-and-mouth disease viruses (FMDV), and human and equine rhinoviruses. We have examined representative viruses from four of these genera for the presence of poly(C) using methods similar to those previously employed with EMCV RNA, in which the poly(C) tract was detected as a very large T_1 RNase digestion product in polyacrylamide gels[1]. All the cardioviruses and FMD viruses examined were found to possess a poly(C) region; we did not detect poly(C) in representatives of the entero- and human rhinoviruses.

The viral RNAs were labelled by cultivating the viruses in medium containing ^{32}P-orthophosphate. Figure 1 shows autoradiographs of polyacrylamide gel slabs in which the products of total T_1 RNase digestion of various ^{32}P-labelled RNAs isolated from the purified viruses were fractionated by electrophoresis. In those cases where very large products (≥ 50 nucleo-

tides) were encountered, the fragments were analysed by further digestion with pancreatic RNase to determine whether they consisted largely of C residues (Table 1). The methods used in the further digestion of the C-rich tracts and in the fractionation and identification of their products are described in the legend to Table 1.

The RNAs of all examples of the cardioviruses and foot-and-mouth disease viruses studied yielded very large products on T_1 RNase digestion, containing mainly C (Table 1). In contrast, none of the six enteroviruses examined gave rise to any such C-rich products, although some seemed to contain A-rich tracts (ref. 5 and our unpublished observations). Similarly, the RNAs of three different rhinovirus strains yielded no large C-rich T_1 RNase digestion products. We have examined only one virus outside the picornavirus family, the togavirus, Semliki Forest virus[6]. T_1 RNase digestion of the virus 42S RNA and the virus-induced 42S and 26S RNAs gave rise to several A-rich products, but no C-rich oligonucleotides (I. Kennedy, A. P., C. N. and P. F., unpublished).

compositions. They each contained about 30–40 more C residues than the poly(C)-containing bands A_0 and M_0, respectively in EMCV and mengovirus RNA (see Table 1).

Finally, the RNAs of the FMDV strains O-V1 and C-GC gave rise to very large T_1 RNase digestion products containing at least 200 nucleotides. Strain C-GC yielded two large C-rich bands with very similar mobilities, apparently in equimolar amounts, which were barely separated in the 10% polyacrylamide gel. These bands (C_A and C_B) were almost identical in electrophoretic mobility to the large C-rich product (OV_B) from O-V1 RNA (Fig. 1). An additional C-rich band (OV_0) was encountered in O-V1 RNA with an electrophoretic mobility identical to that of the poly(C)-containing products from MEV and A-61 RNAs. The compositions of the poly(C) tracts set out in Table 1, however, do not preclude relationships between the larger digestion products of some of these viral RNAs, that is, the smaller products might have arisen by oversplitting of larger ones.

We are uncertain whether the C-rich products from MEV and

Table 1 Occurrence and analysis of C-rich fragments from total T_1 RNase digestion of various viral RNAs

Genus*	Virus	No. of analyses	Large C-rich T_1 RNase digestion products (Fig. 1)	Estimated size (nucleotides)	% C	Pancreatic RNase digestion products
Cardio	EMC	5	A_0	~100	80–90	1AAC, 1AC, 4U, 80–90C, 1G
	Mengo	3	M_0	~100	80–90	1AAC, 1AC, ~4U, 80–90C, 1G
			M_A	~150	> 75	mainly C
	Maus-Elberfeld	3	ME_0	~150	80–90	1AAAAC, 1AAU, 1AAC, ~2AU, ~4AC, ~6U, 90–110C, 1G(?)
Foot-and-mouth disease	A-61	3	A-61_0	~150	80–90	mainly C (some AC and U also present)
	O-V1	3	O-V_0	~150	80–90	$1A_3X$, 1AAC, 1AU, ~4AC, ~8U, 90–110C, 1G(?)
			O-V_B	≥200	70–80	$1A_3X$, $1A_3C$, 1AAC, 5–10AC, 10–15U, ~150C, 1G(?)
	C-GC	2	C_A	≥200	75–85	$1A_3X$, $1A_2C$, 1AU(?), ~10AC, ~17U, ~150C, 1G(?)
			C_B	≥200	75–85	$1A_3X$, $1A_2C$, 1AU(?), ~10AC, ~17U, ~150C, 1G(?)
	C-997	1	not shown	~150	80–90	mainly C

* The RNAs of the following viruses did not give rise to large C-rich T_1 RNase oligonucleotides: the enteroviruses, polio (type 1, ts^+); Echo (type 12); coxsackie Faulkner; coxsackie 8068; bovine entero- and swine vesicular disease: the human rhinoviruses, types IA, 2 and 14: the togavirus, Semliki Forest.

The poly(C)-containing fragments, shown in column 4, were completely digested with pancreatic RNase, and the resultant oligonucleotides (column 7) were separated by electrophoresis on paper, quantitated and identified as previously described[1]. These compositions are calculated from different numbers of experiments (column 3) and enable the lengths of the fragments (column 5) to be estimated. These lengths are consistent with the mobilities of the fragments in 10% polyacrylamide relative to the electrophoretic front (Fig. 1). The C content of the large T_1 RNase-resistant fragments (column 6) is calculated from their pancreatic RNase digestion products. In those cases where there is uncertainty about whether G is molar, the yields of oligonucleotides are calculated assuming AAC is present once.

The large C-rich products arising from the cardioviruses and foot-and-mouth disease viruses shown in Table 1 fall into three size classes. The RNAs of EMCV and mengovirus each yielded products of about 100 nucleotides in length, containing at least 90% C (Fig. 1 and Table 1). It is clear that the nucleotide sequences of these viral RNAs are similar, since not only the Poly(C)-containing products A_0 and M_0, but also the smaller oligonucleotides A_1–A_3 (Fig. 1 and ref. 1) and M_1–M_3 (Fig. 1) gave rise to the same products upon subsequent pancreatic RNase digestion; however, two products were observed in mengovirus RNA which were not observed in EMCV RNA. The first was a large product, M_A, which occurred as a broad, possibly heterogeneous band in polyacrylamide gels (Fig. 1). It contained about 150 nucleotides as judged from its electrophoretic mobility, and from its intensity seemed submolar relative to M_0. Like M_0, the band M_A seemed to contain mainly C (Table 1). Its presence is noteworthy, since it is of similar size to the poly(C)-containing products arising from some of the other viruses examined (see below). The second T_1 RNase product, M_x, found in mengovirus, but not in EMCV, also seemed rich in C (see Table 1). Its composition precludes it arising as a result of oversplitting of M_0.

Maus-Elberfeld virus (MEV) and the FMDV strains, A–61 and C–997, yielded C-rich products of about 150 nucleotides, as indicated both by their electrophoretic mobilities and their

the FMDV strains yielded molar amounts of G on subsequent pancreatic RNase digestion (Table 1). If these products lack G, they might be expected to arise from the 3′-terminus of the viral RNA. At this stage, however, our analyses of these products are insufficiently precise to enable us to discuss this possibility further.

The results described show that the presence of large tracts of poly(C) is not limited to EMCV RNA, but also occurs in the two other cardioviruses and three FMDV types examined. Similar tracts were not found in the RNAs of the other picornavirus genera. But if the poly(C) tract is involved in replication, for example as part of a recognition site for a viral replicase[7], it would be surprising if it is not conserved at least to some extent throughout the Picornaviridae, since the replication processes are believed to be similar for all these viruses[2]. The methods we have used here cannot resolve this question.

It is clear, however, that the grouping of the picornaviruses according to whether or not large C-rich oligonucleotides arise on digestion of their RNAs is in accord with a classification based on their physicochemical properties. For example, our results support the conclusion that the cardioviruses should be regarded as a genus distinct from the enteroviruses[3,8]. It is also of interest that EMCV and mengovirus, originally isolated from primates[9,10], gave identical poly(C) tracts, whereas the closely related Maus-Elberfeld virus (MEV), which was of murine

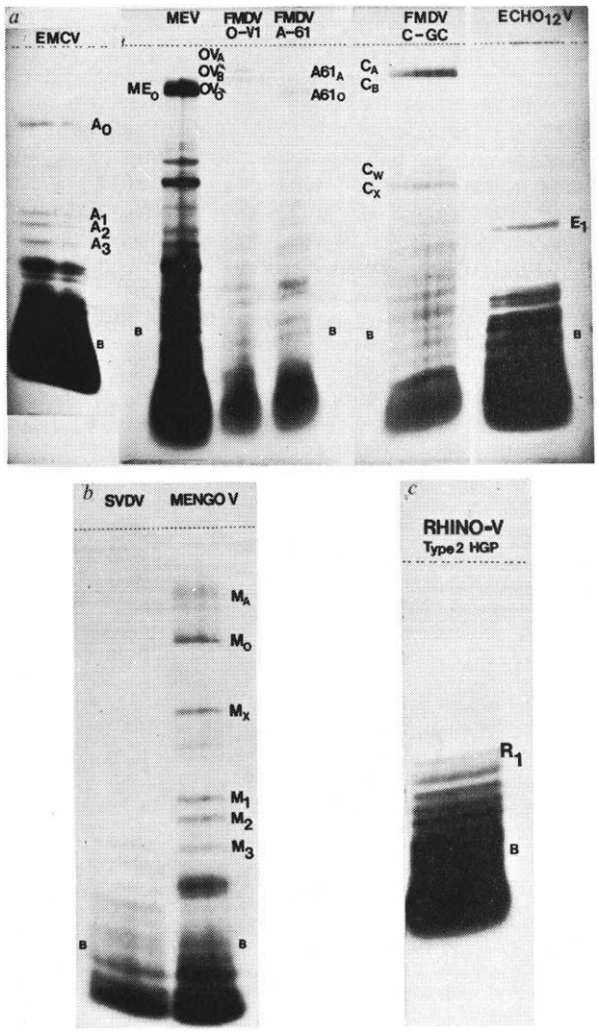

Fig. 1 The following viruses were grown in monolayers of the cell cultures indicated in parentheses in phosphate-free Earle's saline containing ^{32}P-orthophosphate: foot-and-mouth disease virus, strains O-V1, A-61, C-997 and C-GC (BHK-21); the cardioviruses, MEV and mengovirus (BHK-21); bovine enterovirus, VG-5-27 (BHK-21); swine vesicular disease virus, Italy 1/66 (IB-RS-2); coxsackie B5 virus, Faulkner strain and isolate 8068 (HeLa). The viruses were purified according to the method described for foot-and-mouth disease virus[12] and their RNAs extracted with phenol-SDS. Echo virus, type 12 (LLC-MK$_2$); poliovirus type 1, strain ts$^+$ (HeLa S3); and the rhinovirus types 1A, 2 and 14 (L-132) were also grown in monolayers of cell cultures, except that they were purified and their RNAs extracted according to the method used for EMCV, given in ref. 1. Each sample of ^{32}P-labelled viral RNA (0.1×10^6–20×10^6 c.p.m. and containing 50–200 μg unlabelled carrier RNA) was dissolved in 0.02 M Tris-HCl *p*H 7.5; 0.002 M EDTA containing T$_1$ RNase (Sankyo, Japan) at an enzyme:substrate (w/w) ratio of 1:40. Digestion was complete in 1 h at 37° C. The resultant oligonucleotides were fractionated by electrophoresis in 10% polyacrylamide gel slabs as described previously (see Fig. 2 of ref. 1). The radioactive bands were located as shown by autoradiography, and excised using the autoradiograph as a guide. RNA was eluted electrophoretically from each numbered gel band on to DEAE-cellulose paper disks. Subsequent elution of the labelled fragments from the disks was performed using freshly prepared 30% triethylammonium carbonate, *p*H 10.0, as described by Sanger *et al*[13].

origin[11], gave a substantially longer tract of poly(C).

Thus, the occurrence of poly(C) regions in the picornaviruses seems to be genus specific. Furthermore, the lengths of the poly(C) tracts in the RNAs of individual members of the same genus, and even more significantly of subtypes of one serotype of an individual virus, can differ considerably. These findings emphasise the value of this type of analysis in the classification of viruses. Although clearly falling short of what could be achieved by complete nucleotide sequencing, the methods used here could lead to the elucidation of the variations in RNA primary structure which account for strain and serotype differences.

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Two functional states of the RNA polymerases in the rat hepatic nuclear and nucleolar fractions

IN eukaryotic cells, the DNA-dependent RNA polymerase is known to be bound to chromatin[1]. As it is known that chromatin is a restricted template[2,3], the question is whether all or only a fraction of the RNA polymerases bound to the chromatin is functionally active. The experiments reported here provide evidence that only a fraction of the RNA polymerases present in both nuclei and the nucleolar fraction is functionally active on chromatin.

It was noticed previously[4], when nuclei or nucleoli were treated *in vitro* with actinomycin D (10 μg ml^{-1}) to block the endogenous DNA-template function, that the RNA polymerase activity could be measured independently *in situ* in the same reaction vessel with a synthetic template, such as poly(dC) or poly(dA-dT). These synthetic templates are effective in directing RNA synthesis in the presence of actinomycin D, because they do not contain the deoxyguanosine moiety and hence do not react with the actinomycin D (ref. 5). As Fig. 1 shows when endogenous nuclear RNA synthesis was effectively blocked by actinomycin D, the incorporation of ^{14}C–UTP into RNA was an almost linear function of the concentration of poly(dA-dT). Fur-

Table 1 Functional independence of the engaged and free nuclear and nucleolar RNA polymerases

Incubation conditions	RNA polymerase activity pmol of labelled precursor incorporated per mg DNA			
	^{14}C–UTP	%	^{14}C–GTP	%
Nuclei	706	100	943	100
Nuclei + poly (dA–dT)	2,274	322	1,098	116
Nucleoli	995	100	1,535	100
Nucleoli + poly (dA–dT)	2,901	292	1,595	104
Nuclei	866	100	1,057	100
Nuclei + poly (dI–dC)	1,068	123	8,930	845
Nucleoli	6,599	100	11,686	100
Nucleoli + poly (dI–dC)	7,692	116	52,761	451

RNA polymerase activity was assayed as described in the legend to Fig. 1. When indicated, 25 μg poly(dA-dT) or 12.5 μg poly(dI-dC) (Boehringer Mannheim) was used as the exogenous template.

thermore, when nuclear or nucleolar suspensions were incubated *in vitro* in the absence of actinomycin D, the addition of poly(dA-dT) stimulated the incorporation of ^{14}C–UTP into RNA threefold (Table 1). If the RNA polymerases bound to chromatin were all functionally active toward the endogenous chromatin template, then all or part of the RNA polymerase would have to leave the chromatin and transcribe the poly(dA-dT) in order to account for increased ^{14}C–UTP incorporation into RNA after addition of the synthetic template. But, as the right hand columns of Table 1 show, when ^{14}C–GTP was used to monitor RNA synthesis, the addition of poly(dA-dT) did not enhance or antagonise incorporation. These results clearly indicate that the endogenous chromatin template retained all of the functionally active RNA polymerase (engaged form) for ^{14}C–GTP incorporation in the presence

Table 2 Effect of α-amanitin on the engaged and free nuclear RNA polymerase activity measured with endogenous and exogenous template

Incubation conditions	RNA polymerase activity pmol of ^{14}C–UMP incorporated per mg DNA	%	α-Amanitin sensitivity % Inhibition
Nuclei	790	100	
+ α-Amanitin	551	70	30
+ Actinomycin D poly(dA–dT)	931	118	
+ Actinomycin D poly(dA–dT) α-amanitin	657	83	30
+ Poly(dA–dT)	2579	326	
+ Poly(dA–dT) α-amanitin	1244	157	52
+ Actinomycin D	133	17	
+ Actinomycin D α-amanitin	105	13	
Solubilised nuclear enzyme			
+ Native calf thymus DNA	265		
+ Native calf thymus DNA α-amanitin	141		47

Assay conditions were the same as described in Fig. 1. When required, 2 μg α-amanitin (Henley, New York), 10 μg actinomycin D, and 25 μg poly(dA-dT), were added, singly or in combination, to the standard reaction mixture at time zero. Nuclear RNA polymerase was solubilised by the method of Roeder and Rutter[13]. Fraction 4 of the solubilised nuclear enzyme was used for the assay. Calf thymus DNA (25 μg) was used as template. Its activity was expressed as pmol of ^{14}C–UTP incorporated per mg protein.

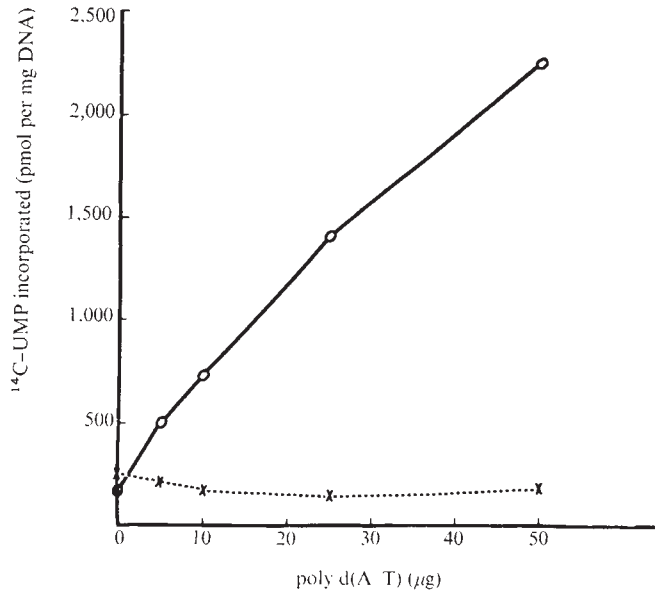

Fig. 1 Male Sprague-Dawley rats, about 200 g body weight, were used. Rat liver nuclei and nucleoli were isolated as described before[4]. RNA polymerase activity was assayed *in vitro* with a standard reaction mixture of 0.65–0.75 ml total volume containing (μmol): Tris-HCl (*p*H 7.9 at 23° C), 50; $MnCl_2$, 1; 2-mercaptoethanol, 14; $(NH_4)_2SO_4$, 40; and ATP, CTP, UTP and GTP, each, 0.1; with 0.1 μCi of either 2-^{14}C–UTP (Schwarz/Mann, specific activity 42 Ci mol^{-1}) or 8-^{14}C–GTP (Schwarz/Mann, specific activity 39 Ci mol^{-1}) as indicated. When required, 10 μg of actinomycin D (Nutritional Biochemicals) and an indicated amount of poly(dA-dT) (Miles) were also added to the incubation mixture at time zero. The reaction was initiated by the addition of 0.1 ml nuclear or nucleolar suspension in 0.34 M sucrose derived from 0.1 and 0.6 g of liver, respectively, and incubated for 10 min at 37° C with shaking. The reaction was stopped by transfer of the reaction tubes to chipped ice, followed by immediate addition of 5 ml of cold 10% trichloroacetic acid containing 1% sodium pyrophosphate. The acid-insoluble material was collected on Whatman GF/C filters and washed seven times with 5 ml of 5% cold trichloroacetic acid containing 1% sodium pyrophosphate, and once with 5 ml of 60% ethanol. The filters were air dried in liquid scintillation vials and counted in 10 ml of Bray's solution[10]. The specific activity of RNA polymerase was expressed as pmol of labelled ^{14}C–UMP or ^{14}C–GMP incorporated per mg DNA. DNA concentrations were determined as described by Burton[11]. Protein was measured by the method of Lowry *et al.*[12] with bovine serum albumin as standard. Nuclei + actinomycin D (×); nuclei + actinomycin D + poly(dA-dT) (○).

of the poly(dA-dT) synthetic template. Consequently, the RNA polymerase population that transcribed the poly(dA-dT) template had to be derived from a population of RNA polymerase, which was functionally inactive toward the endogenous chromatin template (free enzyme). The same conclusion was reached when poly(dI-dC) was used as the exogenous template. Where ^{14}C–GTP incorporation into RNA was stimulated several fold, again the endogenous template function, as indicated by ^{14}C–UTP incorporation data, was not significantly affected by the addition of the synthetic template (bottom part, Table 1).

Several species of RNA polymerases are known to exist in eukaryotic nuclei[6]. Since α-amanitin specifically inhibits nucleoplasmic RNA polymerase II (refs 7–9), it was decided to use it as a probe to determine whether the chromatin bound RNA polymerases in both the 'engaged' and 'free' states represent the same enzyme. The results presented in Table 2 show that both the 'engaged' and 'free' nuclear RNA polymerases were similarly inhibited by α-amanitin. For example, the activity of the 'engaged' enzyme on the

Table 3 Effect of α-amanitin on the engaged and free nucleolar RNA polymerase activity measured with endogenous and exogenous template

Incubation conditions	RNA polymerase activity pmol of ^{14}C–UMP incorporated per mg DNA	%	α-Amanitin sensitivity % Inhibition
Nucleoli	1,434	100	
+ α-Amanitin	1,289	90	10
+ Actinomycin D poly(dA–dT)	2,411	168	
+ Actinomycin D poly(dA–dT) α-amanitin	2,534	177	0
+ Poly(dA–dT)	4,614	322	
+ Poly(dA–dT) α-amanitin	3,928	274	15
+ Actinomycin D	0	0	
+ Actinomycin D α-amanitin	0	0	

Conditions were the same as described in the legend to Table 2.

endogenous chromatin template was inhibited 30% by α-amanitin, whereas the 'free' polymerase, which utilised the exogenous poly(dA-dT) template, was also inhibited 30% under conditions where the endogenous chromatin template activity was inhibited by actinomycin D. In the absence of actinomycin D, the inhibition was 52%. This level of inhibition agrees very well with the inhibition of the solubilised RNA polymerase whch was 47% when calf thymus DNA was used as template.

Table 3 presents data which indicate that this toxin had no effect on both the 'engaged' and 'free' nucleolar RNA polymerases, as has been reported[7–9]. These data suggest that the 'engaged' and 'free' forms of RNA polymerase in nuclei most probably represent the same enzyme, and similarly the 'engaged' and 'free' forms present in the nucleolus also are derived from a single population of RNA polymerase molecules.

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Location of human satellite DNAs on the Y chromosome

THE technique of *in situ* hybridisation of radioactively labelled DNA or RNA to the denatured DNA in metaphase chromosomes provides a powerful and popular method for identifying the chromosomal location of specific repeated DNA sequences[1,2]. In man, this technique has been used to map the ribosomal cistrons (ref. 3 and H.J.E., M. L. Pardue, and R.A.B., in preparation) and to examine the chromosomal location of three of the four known satellite DNAs[4–8]. Satellite II (ref. 6) was found in the centromeric heterochromatic regions (C band) of chromosome 1 and in the C bands of one C group (chromosome 9) and one E group chromosome pair (tentatively identified as chromosome 16). Satellite III (ref. 8) was found mainly in the C band of chromosome 9, with some in the centromere/short arm regions of the D and G group chromosomes, and possibly in the C bands of chromosomes 1 and 16. The satellite DNA investigated by Saunders *et al.*[7] (which is probably identical to satellite IV of Corneo *et al.*[5]) was only positively identified in the C band of chromosome 9, although other regions of hybridisation were noted. In all these studies on satellite DNAs, the autoradiographic observations were limited because no general chromosome identification was possible. Thus, only major sites of hybridisation could be detected and in some instances the locations of these sites were in doubt.

We have now investigated the chromosome location of the four human satellite DNAs by *in situ* hybridisation using dual karyotype analysis (ref. 9 and J.R.G., R.A.B., R. P. Clayton and H.J.E., in preparaton) (see legend to Fig. 1). This involves quinacrine fluorescence and karyotype analysis of the chromosomes, followed by *in situ* hybridisation and comparison of the autoradiographs with the corresponding fluorescence karyotype. The method allows unequivocal identification of each site

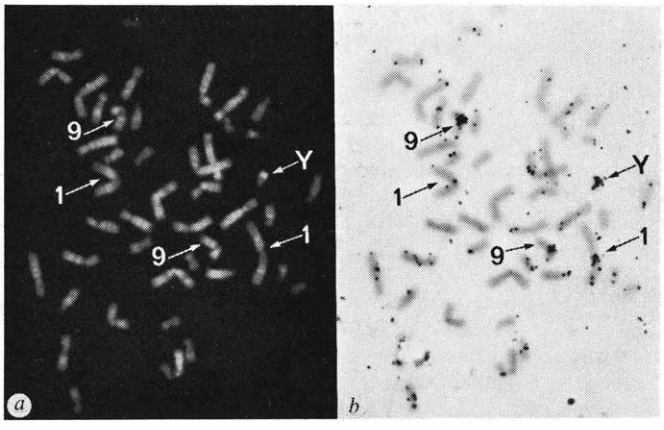

Fig. 1 DNA was prepared from placentae of male babies by the method of Marmur[11]. Satellite DNAs were isolated by centrifugation in Ag^{+}–Cs_2SO_4 or Hg^{2+}–Cs_2SO_4 density gradients[4,5] and buoyant densities and purity checked in neutral CsCl gradients. ^{3}H-labelled cRNAs were prepared *in situ* hybridisation performed as described elsewhere (J.R.G., R.A.B., R. P. Clayton and H.J.E., in preparation). The cRNAs were hybridised to blood lymphocyte chromosome preparations from various normal, and chromosomally abnormal males and females. Chromosomes were first stained with quinacrine and selected well spread metaphase cells showing good Q banding under fluorescence photographed. Following removal of the stain by washing, hybridisation, autoradiography and staining with Giemsa or Crystal Violet, these cells were again located, re-photographed and dual karyotypes constructed (ref. 9 and J.R.G., R.A.B., R. P. Clayton and H.J.E., in preparation). *a*, Fluorescence photograph of quinacrine stained chromosomes before *in situ* hybridisation. *b*, Giemsa stained autoradiograph of same cell after hybridisation with cRNA prepared from DNA satellite II. Note the concentration of grains over both chromosome 9 and the Y chromosome and that the differences in grain count between homologous chromosomes of pairs 1 and 9, parallel differences in the sizes of their respective C bands.

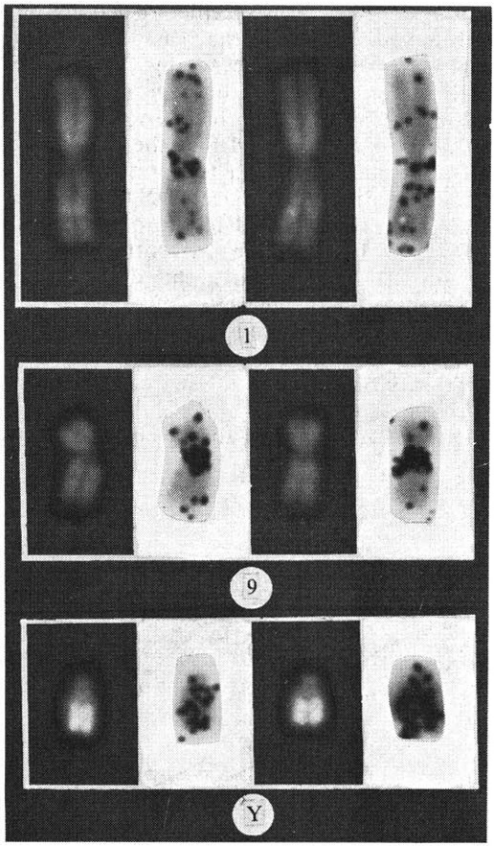

Fig. 2 Partial dual karyotype of chromosomes (1, 9 and Y) prepared from a blood lymphocyte of a 47,XYY male and hybridised with cRNA prepared from DNA satellite IV. All methods were as described in the legend to Fig. 1.

of hybridisation. Full details of these methods and results will be published elsewhere (J.R.G., A.R.M., R.A.B., R. P. Clayton, and H.J.E., in preparation), but we would like to comment here on one important aspect of our findings.

Although our results differ in several minor respects from those previously published, one major location of satellites II, III and IV seems to have been completely overlooked. We find that a substantial portion of all three of these satellites, and also of satellite I, is located in the human Y chromosome, in particular in the brightly fluorescent region of the long arm (Fig. 1)—the region that stains intensely with Giemsa with the C-banding technique. This specific hybridisation to the Y chromosome is a consistent finding, made in numerous experiments using cells from normal males and from males with a 47,XYY chromosome constitution. In the latter case, hybridisation is found on both Y chromosomes and an example is illustrated in Fig. 2.

The apparent discrepancy between our finding and those previously published cannot be accounted for by errors in observation, since the Y chromosome can be identified without recourse to fluorescence analysis, and the amount of cRNA hybridised is large. Saunders *et al.*[7] hybridised cRNA to cultured fibroblast metaphase cells and there is no mention of the Y chromosome or the sex of the donor(s) of these cells. The cRNAs from satellites II and III, however, were hybridised to cells containing a Y chromosome, but no specific hybridisation to the Y was noted[6,8]. The difference is unlikely to be in the origins of the template DNAs used by the different groups, but may be a consequence of differences in techniques used in the hybridisation process.

We have found that the Y chromosome contains a considerable proportion of repetitious DNA, and that some of this is in a satellite-free fraction (J.R.G., R.A.B., R. P. Clayton, and H.J.E.; J.R.G. and A.R.M., in preparation). The present studies show that the human Y chromosome also contains repetitious sequences from all four of the human satellite DNA fractions. Previous cytochemical results have suggested that the human Y chromosome may contain highly repetitious DNA[10], but this is the first direct demonstration of this fact.

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Murine leukaemia virus RNA transcription from chromatin of normal and infected BALB/c spleen

Studies on Rous sarcoma virus (RSV), murine leukaemia virus (MuLV) and mammary tumour virus (MTV) systems have shown that at least parts of viral genomes are present in multiple copies in the natural host DNA. RSV-specific nucleotide sequences have been detected in various chick cells, including Rous sarcomas and embryos with or without RSV gs antigens and chick helper factor[1,2]. Similarly, normal and virus-transformed mouse and rat cells contained equivalent amounts of MuLV-specific DNA sequences[3]. MTV-specific DNA genomes have been detected in equal numbers in mouse strains with both high and low incidences of mammary tumours[4]. In addition, induction of leukaemia viruses from apparently uninfected mouse and chicken cells indicates that complete, 'endogenous' viral genomes are present in these cells[5-8]. But the integration of 'exogenous' viral genomes, upon infection with an oncogenic RNA virus, cannot be proved in an homologous system because of the lack of specific markers which could distinguish between exogenous and endogenous MuLV genes. Actual integration was demonstrated in the case of some heterologous systems, upon infection of duck and of mouse cells with RSV[9].

Evidence exists that partial transcription of MuLV genomes occurs in a virus-free cell line, but to a lesser extent than in some of its transformed derivatives[10]. A similar observation was reported in the case of MTV-RNA transcripts in tumours, lactating mammary glands, spleens and livers from high and low incidence mouse strains[11]. More direct evidence for transcription of integrated viral genes was obtained by studying DNA-containing tumour virus systems. Polycistronic RNA molecules containing both cellular and viral base sequences were reported in adenovirus and in simian virus 40 (SV40) transformed cells[12,13]. Moreover, Astrin[14] and Shih *et al.*[15] demonstrated that chromatin isolated from SV40-transformed mouse cells can synthesise SV40 RNA transcripts *in vitro*. Here

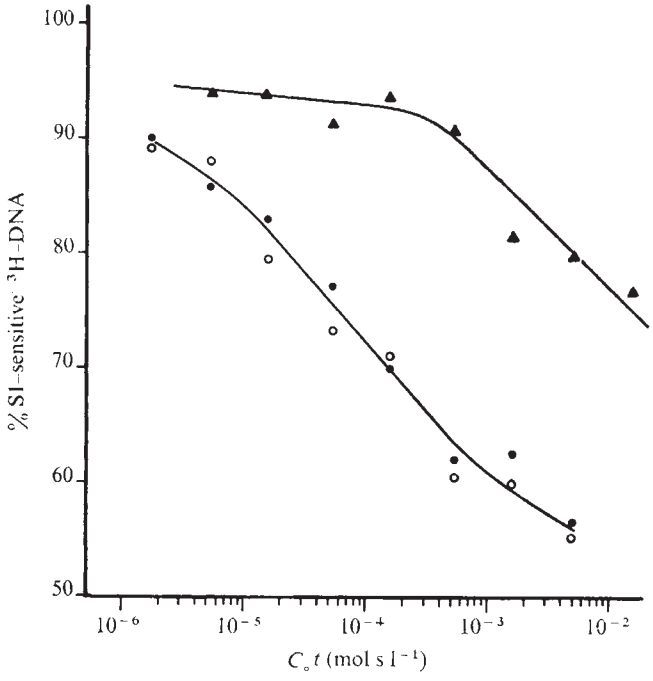

Fig. 1 Annealing curves of RLV–^{3}H–DNA in the presence of 15 mg ml^{-1} of either salmon sperm DNA (▲), normal BALB/c spleen DNA (●), or RLV-infected BALB/c spleen DNA (○). RLV was obtained from Electronucleonics. The virus (approximately 1 mg protein) was incubated for 18 h at 37° C in a 8 ml standard reaction mixture and the ^{3}H–DNA product was purified as described[21]. Non-radioactive DNA was extracted from isolated spleen nuclei of normal and RLV-infected BALB/c mice. The extraction procedure was essentially that of Marmur[22]. It is included two 30 min incubations at 37° C in the presence of 100 μg ml^{-1} RNase and two 30 min incubations at 37° C in the presence of 400 μg ml^{-1} pronase. Salmon sperm DNA (Sigma) was treated with RNase and pronase, and deproteinised before use. DNA fragments were prepared by sonication (Mullard sonifier) and their molecular weight, as determined by analytical ultracentrifugation (Beckman model E), ranged from 130,000 to 160,000 daltons. The DNA samples to be annealed were denatured for 15 min at 100° C in 30 mM Tris, *p*H 7.6. After rapid chilling at 0° C, NaCl was added to a final concentration of 0.6 M. Fifteen microlitre duplicate samples, containing each 4,500 d.p.m. of viral ^{3}H–DNA (Sigma). One of the duplicate samples contained 80 ng 67° C in sealed glass capillaries (Drummond microcaps) for periods of time ranging from 1 min to 6 d. After incubation, the content of each capillary was chilled and poured into 0.6 ml 30 mM sodium acetate, *p*H 4.6, 50 mM NaCl, 1 mM $ZnSO_4$, 5% glycerol and 50 μg ml^{-1} native thymus DNA (Sigma). One of the duplicate samples contained 80 ng ml^{-1} (8 activity units ml^{-1}) of *Aspergillus oryzae* SI-nuclease (prepared according to ref. 23). The mixtures were incubated for 15 min at 45° C, chilled, precipitated in the cold by 1.2 ml 10% perchloric acid (PCA) and filtered through Whatman GF C filters. The filters were washed with 10 ml 5% PCA, dried under a heat lamp and counted in a Packard scintillation counter. The percentage of reannealed ^{3}H–DNA refers to the ratio of SI-nuclease-resistant radioactivity to the total radioactivity in the samples that were not submitted to SI-nuclease treatment. No loss of total PCA-precipitable radioactivity was observed after incubation periods up to 6 d. The results presented in the graphs were not corrected for the presence of nonreannealing ^{3}H–DNA present in the viral product.

we report that MuLV-DNA copies are present in equal amounts in spleen DNA from both normal mice and mice infected with Rauscher leukaemia virus (RLV), but that the viral genomes are expressed to a much larger extent in leukaemia mouse spleen chromatin than in normal spleen chromatin.

RLV-infected BALB/c mice were killed 15 d after virus inoculation. Infected and non-infected spleens were both processed in the same way for DNA extraction, as described in the legend to Fig. 1. The unlabelled cellular DNAs were tested for virus-specific nucleotide sequences by their capacity to accelerate the reassociation kinetics of labelled DNA, synthesised *in vitro* by partially disrupted RLV virions. The apparent reduction in the C_0t values (product of initial concentration of DNA, in mol of nucleotide per l, and time (s)), showed that the reassociation rate of the ^{3}H-DNA product was, in the presence of either infected or uninfected BALB/c spleen DNA, increased about 300-fold over the rate observed in the presence of salmon sperm DNA (Fig. 1). At these concentrations of cellular DNA and viral ^{3}H-DNA product, this degree of acceleration is consistent with the presence of about 40 copies of viral sequences per diploid mouse genome. This result is in good agreement with those of Gelb *et al.*[3], who reported 50 leukaemia virus-specific DNA copies per diploid BALB/3T3 cell.

Subsequently we determined the level of *in vivo* expres-

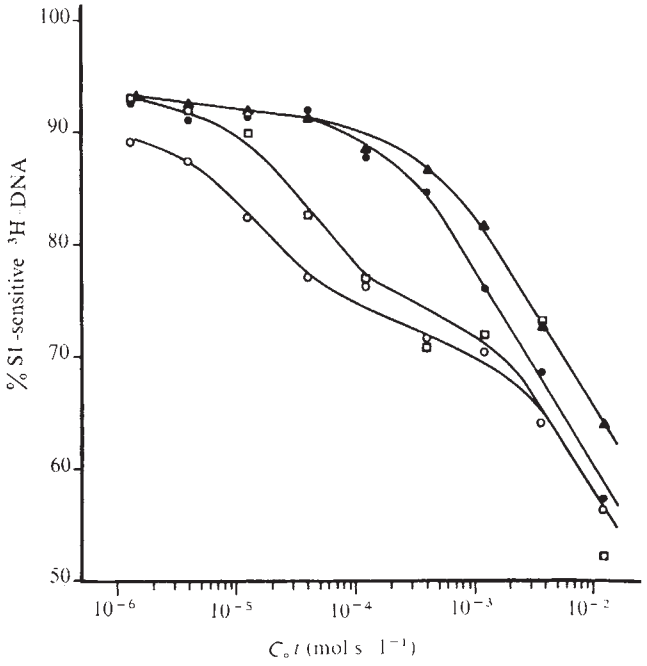

Fig. 2 Annealing curves of RLV–^{3}H–DNA in the presence of 1.4 μg ml^{-1} 70S RLV-RNA (□), 200 μg ml^{-1} endogenous RNA from normal BALB/c spleen chromatin (●), 600 μg ml^{-1} endogenous RNA from RLV-infected BALB/c spleen chromatin (○), or no chromatin nor viral RNA (▲). High molecular weight (70S) RLV-RNA was purified from infected plasma virions according to ref. 24. Chromatin was purified from spleens as described[25], except that the ultimate centrifugation step was omitted to avoid the possible loss of a preferential DNA fraction. It was suspended at a concentration of 150 μg DNA ml^{-1} in 50 mM Tris, *p*H 8.0. NaCl and SDS were added to a final concentration of 0.4 M and 1%, respectively. Deproteinisation was performed on 100 ml volumes (15 mg chromatin DNA) at room temperature by two extractions with an equal volume of phenol-cresol (7:1) and one extraction with chloroform-isoamyl alcohol (24:1). Nucleic acids were ethanol-precipitated, dissolved in 6 ml 10 mM Tris, *p*H 7.6, 0.1 M NaCl and 5 mM $MgCl_2$, and incubated for 60 min at 37° C after addition of 250 μg DNase I (Sigma). Deproteinisation in the presence of SDS and NaCl, and ethanol precipitation were repeated as before. The resulting precipitate was suspended in a minimal volume of 10 mM Tris, *p*H 7.6, and the sample was passed over a 1.6 cm × 100 cm Sephadex-G-50 (medium) column equilibrated with 10 mM Tris, *p*H 7.6. The RNA eluting in the excluded volume was ethanol-precipitated and used for annealing experiments with viral ^{3}H-DNA. The annealing reactions were performed in 20 μl duplicate samples containing 4,500 d.p.m. RLV-^{3}H-RNA, 200 μg yeast RNA (Sigma) and RNA to be tested. The procedure was the same as for annealing in the presence of DNA, except that the SI-nuclease reaction mixtures contained 150 μg ml^{-1} calf thymus DNA and that precipitation was performed with TCA (trichloroacetic acid) instead of PCA.

sion of the viral DNA genomes in chromatin isolated from normal and RLV-infected BALB/c spleens. Chromatin from both normal and infected spleens was purified and processed for RNA extraction as described in legend to Fig. 2. RLV-^{3}H-DNA was again used as a probe for virus-specific nucleotide sequences in chromatin RNA. High molecular weight (70S) RLV-RNA was used as a viral RNA standard. Leukaemic spleen chromatin RNA accelerated the reassociation kinetics of the probe ^{3}H-DNA to a much higher degree than did uninfected spleen chromatin RNA (Fig. 2), but only over a limited C_0t range. The latter observation might be explained by the possible presence of nonviral nucleotide sequences in the ^{3}H-DNA product. The acceleration rates, when compared with the acceleration effect of the 70S viral RNA, are consistent with the presence of 0.7 and 0.06% viral sequence in the RNA population of leukaemic and normal spleen chromatin, respectively.

Endogenous chromatin RNA might consist mainly, if not exclusively, of newly *in vivo* synthesised molecules. If so, *in vitro* transcription of chromatin by RNA polymerase would not result in a measurable enrichment of the total RNA population in virus-specific sequences. On the contrary, chromatin might contain appreciable amounts of associated RNA, considered as a distinct, structural and/or regulatory component, which does not seem very likely[16–18]. In the latter case, the *in vitro* RNA transcripts would contain a higher proportion of viral sequences than the endogenous chromatin RNA. Our experiments favoured the first hypothesis: we incubated infected and uninfected spleen chromatin in the presence of all four unlabelled ribonucleoside triphosphates. Bacterial enzyme was used because, besides the fact that it is easier to prepare in large amounts, it displays a good fidelity in chromatin transcription[14]. The incubation resulted in a fivefold increase of the RNA content in the reaction medium. This RNA accelerated the reassociation of the probe ^{3}H-DNA to the same degree as did the corresponding, endogenous chromatin RNA. In the case of RNA from leukaemic spleen chromatin, the acceleration occurred throughout the observed C_0t values, in contrast with the effect of either 70S RLV-RNA or endogenous leukaemic spleen RNA. This observation suggests that *in vitro* chromatin transcription yields RNA sequences that are not present, either in the endogenous chromatin RNA population, or in the high molecular weight RLV-RNA. These additional sequences are, however, complementary to the ^{3}H-DNA probe. Hence the probe should be considered as only partially virus specific.

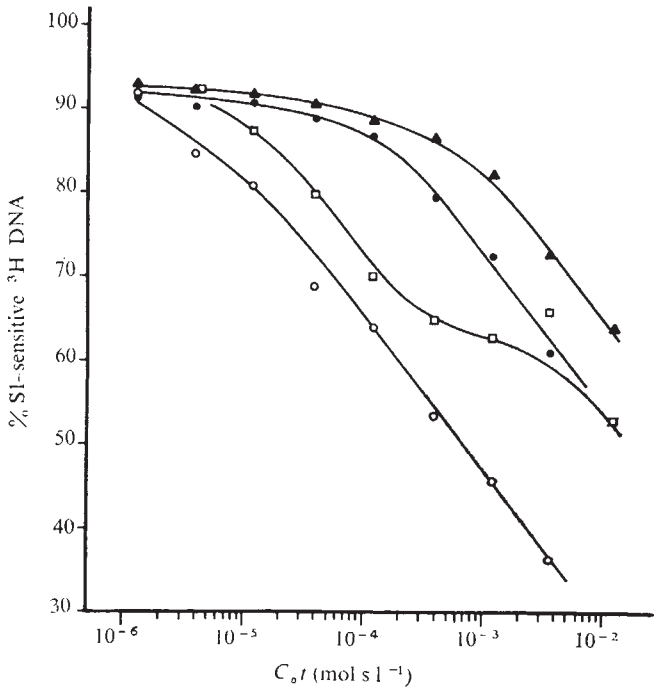

Fig. 3 Annealing curves of RLV-^{3}H-DNA in the presence of 1.4 μg ml^{-1} 70S RLV-RNA (□), of 1.1 mg ml^{-1} *in vitro* RNA transcripts from normal BALB/c spleen chromatin (●), of 1.3 mg ml^{-1} *in vitro* RNA transcripts from RLV-infected BALB/c spleen chromatin (○), or neither chromatin nor viral RNA (▲). *E. coli* RNA polymerase was purified according to Burgess[26], using the alternate method of gel filtrations on Bio-Gel A-5m and Bio-Gel A-1.5 m columns. *In vitro* synthesis of RNA was performed as follows: a 100 ml reaction mixture, containing 50 mM Tris, *p*H 8.0, 100 mM NaCl, 4 mM $MgCl_2$, 1 mM $MnCl_2$, 0.1 mM DTT, 0.2 mM of each unlabelled ATP, CTP, GTP and UTP, 150 μg ml^{-1} DNA in the form of chromatin, and 150 μg ml^{-1} RNA polymerase, was incubated for 45 min at 37° C. The reaction was terminated by addition of NaCl and SDS to a final concentration of 0.4 M and 1%, respectively. All subsequent operations were performed as described in legend to fig 2.

This work clearly demonstrates that endogenous chromatin RNA from RLV-infected spleens, as well as *in vitro* RNA transcripts from the same spleen chromatin, contains very significantly more virus-specific sequences than the corresponding RNAs from the non-infected spleen chromatin. Thus, our experiments provide direct evidence that the viral genomes, present in the natural host DNA, are largely expressed in RLV-infected spleen. A much lesser degree of expression occurs in the normal tissue.

Whether the transcription from leukaemic spleen chromatin occurs at the level of pre-existing or of newly integrated viral genes cannot be discussed here because of the lack of specific markers. Finally, we feel that our experimental approach is more direct than those consisting of the detection of viral sequences in cytoplasmic[11,19], polyribosomal[20] or even nuclear[11] RNA populations.

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Detection of cell-dependent cytotoxic antibody to cells infected with herpes simplex virus

THE killing or damage of virus-infected cells by immune processes may play a significant role either in host defence or in the production of pathological lesions during virus infections[1]. These immune processes are believed to be directed at virus-induced antigens on the membranes of infected cells. On the basis of *in vitro* experiments, several mechanisms have been proposed as possibly operative *in vivo*. These include the cytolytic action of antibody and complement[2,3], the interaction of antibody, complement and leukocytes[4], and the destruction of infected cells by immune lymphocytes[5–7]. Another mechanism described in non-viral systems is the cytotoxicity demonstrated by the synergistic effect of antibody and nonimmune effector cells[8–10]. The following experiments demonstrate a similar process operative with viruses in which heat-inactivated immune serum augments the damage of herpes simplex virus-infected cells *in vitro* by human mononuclear cells.

Blood from human donors, whose sera contained or lacked neutralising antibodies[11] to herpes simplex virus type 1 (HSV-1), was used. Mononuclear cells (MC) were purified from heparinised blood by centrifugation on a layer of Ficoll-Hypaque[12], washed four times in Hanks balanced salt solution (BSS), counted and diluted in Eagle's growth medium containing 10% inactivated foetal calf serum (EGM). Ninety-five per cent of the cells were mononuclear, about 90% of which were lymphocytes; cell viability exceeded 90%. All human or rabbit sera to be tested were heat inactivated at 56° C for 30 min. The γ-globulin and purified human IgG described below were not heat inactivated.

Table 1 Absorption of augmenting factor by HSV-1-infected BHK-21 cells

Added to MC	Absorption with	% cytotoxicity	% augmentation
PHI 1/500	Not absorbed	36.1±1.8	22.8
PHI 1/500	HSV-1-infected BHK	13.4±1.2	0.1
PHI 1/500	Control BHK	32.1±1.7	18.8
Culture medium	- - -	13.3±2.8	0.0

2.0 ml of a 1/50 dilution of pooled HSV-1 immune serum (PHI) in BSS was mixed with an 0.3 ml pellet of either BHK-21 cells previously infected with HSV-1 or control BHK cells. The mixture was agitated for 120 min at room temperature and then centrifuged. The resulting supernatant was absorbed with a second pellet of infected or control cells for 24 h at 4° C and centrifuged before testing for the presence of augmenting factor. Augmentation is defined as the difference between MC+test substance and the MC control (line 4).

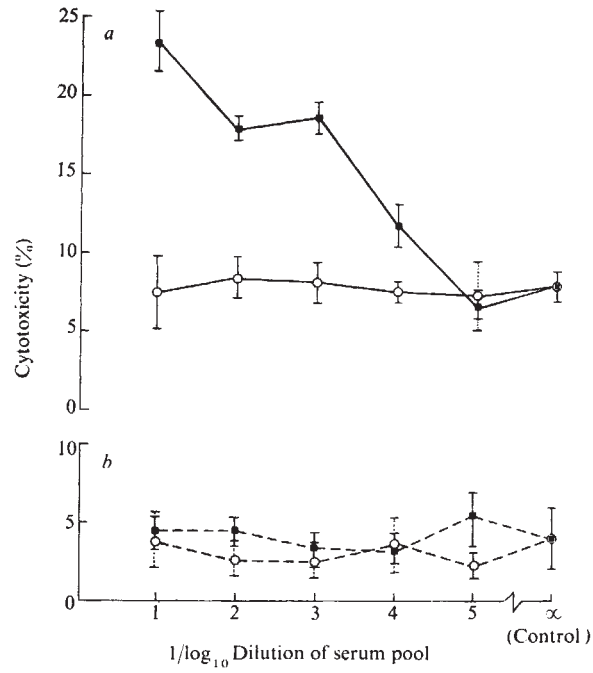

Fig. 1 Augmentation of cell-mediated cytotoxicity by antibody-positive pooled human sera. MC plus dilutions of the seropositive pool (■) are compared with MC plus dilutions of the seronegative pool (○). 5 × 10⁵ human MC (obtained from a seropositive donor) with or without positive or negative serum were added per well. An effector: target cell ratio of 50:1 was found to be optimal in separate experiments. The positive and negative pools comprised sera taken from five positive and negative donors, respectively. *a*, HSV-1-infected HEp-2 target cells; *b*, uninfected HEp-2 target cells.

HEp-2 cells (3×10^6) were exposed simultaneously to 100 μCi ^{51}Cr and 3×10^6 plaque-forming units (PFU) of the Heard strain of HSV-1 in a final volume of 1.0 ml of Eagle's maintenance medium containing 2% inactivated foetal calf serum (EMM). Cells were incubated for 150 min at 37° C and washed four times; 1×10^4 cells diluted in EMM were then added to each well of a Falcon Micro Test II plate. Control HEp-2 cells were treated in a similar fashion except that no virus was added. The sealed plates were incubated at 37° C in a CO_2 incubator and used 24–32 h later when approximately 50% of the infected cells showed cytopathic effects (CPE). At this time, the EMM was removed from each well by aspiration with a Pasteur pipette, and discarded. MC, sera or EGM, alone or in combination, in a total volume of 0.25 ml, were added at 37° C for another 8–12 h, unless stated otherwise (see below and Fig. 2). The bathing medium was then aspirated carefully with a Pasteur pipette, the monolayer lysed with 1 M NaOH, and the medium and cell lysate transferred to tubes for counting separately in a Nuclear–Chicago gamma counter. All determinations were performed in quadruplicate. Cytotoxicity was defined, following the practice of Holm and Perlmann[13], as the percentage of ^{51}Cr release observed with lymphocytes and other immune factors less the percentage ^{51}Cr released spontaneously from HSV-1-infected or control HEp-2 cells.

During the 8–12 h incubation after the addition of MC and serum, the CPE in the infected cultures increased to involve approximately 90% of the cells. Nevertheless, most cells remained attached to the well surface for at least another 12 h. Assessment of cell viability after the 12 h incubation revealed that only ~15% of the attached cells stained with Erythrocin B as compared with ~5% of the cells in the control cultures. During this incubation, infected and control HEp-2 cells showed 17–37% (mean 29)

Table 2 Titration of human commercial γ-globulin and purified IgG

Added to MC	Dilution	Infected cells % cytotoxicity	Infected cells % augmentation	Control cells % cytotoxicity	Control cells % augmentation
γ-globulin	1/500	45.1±2.9†	26.2	12.3±1.2	−2.3
	1/5,000	43.0±2.4†	24.1	16.2±2.3	1.6
	1/50,000	36.0±0.8†	17.1	15.7±3.0	1.1
	1/500,000	21.5±2.2	2.6	19.4±2.8	4.8
	1/5,000,000	22.7±1.9	3.8	17.4±2.4	2.8
	1/50 000,000	20.8±2.1	1.9	14.4±2.2	−0.2
Culture medium	- - - -	18.9±3.5	0.0	14.6±4.5	0.0
IgG	1/1,500*	34.2±4.1†	13.7	12.3±1.3	−0.7
	1/15,000*	25.9±2.5‡	5.4	13.9±0.6	0.9
	1/150,000*	22.9±2.4	2.4	12.6±1.7	−0.4
	1/1,500,000*	21.2±1.2	0.7	12.8±1.3	−0.2
Culture medium	- - - -	20.5±2.8	0.0	13.0±1.0	0.0

Hyland γ-globulin (TA No. 0442R011A7), dialysed to remove preservative, and lyophilised human IgG (Mann, V-3122) were tested. In addition to IgG, the γ-globulin contained 1.7 mg ml^{-1} of IgM as assessed by radial immunodiffusion[14]. At 4 mg ml^{-1}, the IgG material gave only the typical arc of IgG on immunoelectrophoresis against rabbit anti-human serum; it contained no IgM by radial immunodiffusion (sensitivity < 8 μg ml^{-1} IgM). Neither γ-globulin nor IgG in the absence of MC was more cytotoxic than culture medium for the infected target cells.

* Dilution is expressed in relation to the usual IgG concentration of pooled normal adult sera of about 11.5 mg ml^{-1}.

† Significantly different from culture medium control, $P<0.001$.

‡ $P<0.01$.

§ $P<0.05$.

and 16–30% (mean 22) spontaneous ^{51}Cr release, respectively; within a given experiment, infected cells showed 5–8% more background ^{51}Cr release than the control cells. Centrifugation (1,500 r.p.m. for 15 min) of the aspirated bathing medium taken from the infected cultures, however, revealed that about 10% of the total c.p.m. were sedimentable, as compared with 3% for control cultures. Therefore the increased ^{51}Cr release of the HSV-1-infected cells appeared to reflect an increment in the relatively small number of loose or dead cells shed into the aspirated medium rather than an increased loss of ^{51}Cr-labelled cellular macromolecules.

Figure 1 presents data typical of four experiments. Cell-mediated cytotoxicity was augmented by the presence of pooled HSV-1 antibody-positive human serum but not by pooled serum with no detectable HSV-1 antibodies. The increments in cytotoxicity were apparent at dilutions 1/10 to 1/10,000 and were statistically significant at $P<0.001$ (Student's t test). The serum pools alone (without MC) had no effect on ^{51}Cr release. The increment due to the addition of serum was reproducible but varied in magnitude

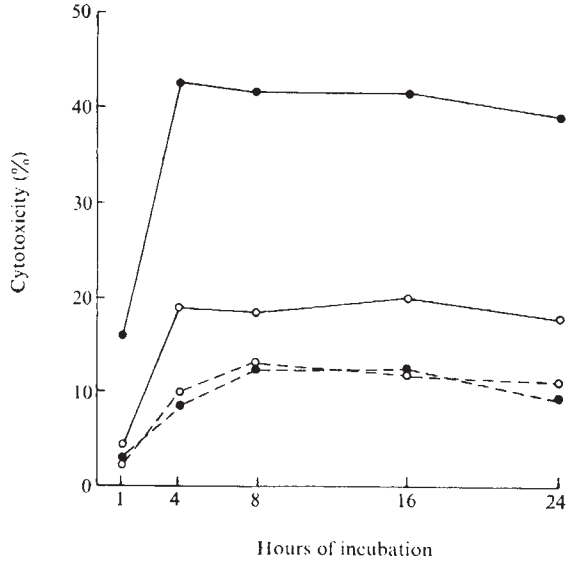

Fig. 2 Effect of time of incubation on augmented cytotoxicity. ———, HSV-1-infected cells; - - - -, control cells; ●, mononuclear cells (from a seropositive donor) (5×10^5 per well and 1/500 antibody-positive serum; ○, mononuclear cells alone.

from experiment to experiment (range 8–30%). In separate experiments, rendering the aspirated bathing medium cell free by centrifugation did not reduce the difference in leakage observed between infected HEp-2 cells incubated with MC and positive serum and infected HEp-2 cells incubated with MC alone. This implies that the augmenting effect of positive serum was on ^{51}Cr release *per se* and not on the sloughing of cells into the culture medium. There was also a small but consistent cytotoxic effect of MC alone on the infected as compared with the control target cells (Fig. 1). The results from additional experiments showing that this effect can be obtained with MC from either seropositive or seronegative donors, however, would indicate that cell-mediated cytotoxicity conveyed by sensitised effector cells alone is not involved.

At a 1/500 dilution of the positive serum, a dilution at which no cytolytic (complement-dependent) antibody could be detected, the augmented cytotoxicity was readily apparent 1 h after the addition of the MC and was maximal at 4 h (Fig. 2). In subsequent study of this phenomenon, an 8–12 h incubation was used.

Nine human immune sera and eight nonimmune sera were tested individually at a 1/500 dilution for augmentation of cytotoxicity. All sera containing HSV-1 neutralising antibody augmented cytotoxicity; in contrast the sera with no detectable HSV-1 antibody failed to do so. In addition, serum from an HSV-1-immunised rabbit showed augmentation, whereas nonimmune rabbit serum did not. The ten immune sera showed augmentation of 11.0 ± 1.8% compared with 0.5 ± 1.3% for the nonimmune sera ($P<0.001$).

The augmenting factor could be removed by absorption of antibody-positive human serum with HSV-1-infected BHK-21 cells but not by uninfected BHK-21 (Table 1). It was also present in high titre both in pooled human γ-globulin (1/50,000) and in highly purified pooled human IgG (1/15,000) (Table 2). These findings taken together strongly suggest that the factor is an antibody of the IgG class.

The fact that the augmenting antibody was found not only in heat-inactivated serum, but at serum dilutions far in excess of those detected by complement-dependent cytolytic antibody assay (not greater than 1/100 in our laboratory), indicated that the classical complement sequence is not necessary for expression of activity. The participation either of complement proteins produced by the MC in our assay system[15,16] or of an alternative pathway using complement components[17] cannot, however, be excluded. It is

interesting that Wunderlich *et al.*[18] found that addition of exogenous human complement does not enhance antibody-dependent cell-mediated cytotoxicity (ADC) in an allogeneic target cell system.

Because ADC has been shown in other systems to be mediated by effector cells obtained from either nonimmune or immune individuals[8], it was interesting to compare the degree of augmentation by MC from HSV-1 immune and nonimmune donors. In the presence of a 1/500 dilution of pooled γ-globulin, cells obtained from five seropositive human donors and three seronegative donors showed 12.4±4.6% and 13.3±2.2% augmentation, respectively. Thus, the presence or absence of neutralising antibodies in an individual does not appear to influence the ability of his or her mononuclear cells to demonstrate the augmentation phenomenon.

The effector cell in our heterogeneous Ficall-Hypaque-purified MC population is unknown. The similar cytotoxicity of nonimmune and immune mononuclear cells in our system as well as the augmentation rather than blocking of cytotoxicity by immune serum suggest that it is not the T lymphocyte. Furthermore, in a nonviral system, the effector cells for ADC in the rat are thymus-independent[19]. The monocyte[20], an adherent cell, is a possibility; however, in other models of ADC, the effector cell is a nonadherent cell[19,21], and preliminary experiments in our laboratory also indicate that the effector cell in our system is nonadherent to a plastic surface. The likely non-adherent effector cell, therefore, probably is a B lymphocyte or the recently described null lymphoid cell[22]. Attempts are now being made in our laboratory to identify the specific effector cell.

The cytotoxic effect of mononuclear cells and immune serum on HSV-1-infected cells is similar to the synergistic or antibody-dependent cytoxicity models described for unsensitised or antigen-coated chicken erythrocytes[8], Chang liver cells[9], allogeneic cells[18,23] and animal tumour cells[10]. These findings therefore extend the possible role of serum arming factors[10] to the immune damage of cells undergoing acute virus infection.

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Cyclic GMP stimulates lymphocyte nucleic acid synthesis

AGENTS that trigger proliferation of lymphocytes[1] and other cultured cells[2] have also been reported to stimulate increases in intracellular guanosine 3′,5′-monophosphate (cyclic GMP). These observations raise the interesting possibility that endogenous cyclic GMP acts as the intracellular mediator of some of the effects of mitogenic agents: if so, then it might be possible to demonstrate experimentally that cyclic GMP, introduced exogenously, has a mitogenic effect. We tested this possibility in mouse splenic lymphocytes and compared the mitogenic effects of cyclic GMP and two of its synthetic derivatives, 8-bromo-cyclic GMP (8-Br cyclic GMP) and N^2 O^2-dibutyryl-cyclic GMP (dibutyryl cyclic GMP), with that of concanavalin A (con A), a mitogenic lectin reported[1] to increase the cyclic GMP content of human lymphocytes. Our results indicate that, like con A, cyclic GMP and its derivatives can stimulate RNA and DNA synthesis in lymphocytes.

Splehic leukocytes from BALB/c mice were prepared as previously described[3] and cultured in serum-free RMPI-1640 medium, at 37° C in an atmosphere of 5% CO_2, 95% air. After addition of nucleosides or con A to the cultures, stimulation of

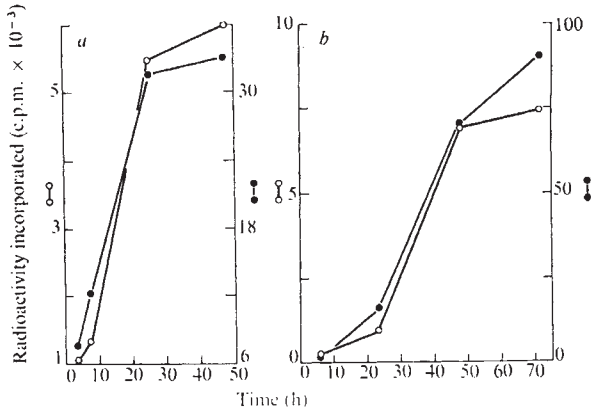

Fig. 1 Time course of incorporation of (*a*) ^{3}H-uridine (26 Ci mmol^{-1}, New England Nuclear Corp.) and (*b*) ^{3}H-thymidine (15 Ci mmol^{-1}, New England Nuclear Corp.) into acid-precipitable macromolecules of spleen cells exposed to 8-Br cyclic AMP (Sigma), 1 × 10^{-3}M (○), or con A (Miles-Yeda), 0.5 μg ml^{-1} (●). Cells (2 × 10^6 per tube) were incubated in 0.5 ml RPMI-1640 medium (Grand Island Biological Co.). At the times indicated on the abscissa, 2 μCi of ^{3}H-labelled precursor was added to the culture and incubation was continued for an additional hour (for uridine) or 2 h (for thymidine); then 2.5 ml 5% TCA was added. The cells were centrifuged and washed two times with 5% TCA, then dissolved in 0.2 ml NaOH, and counted in liquid scintillation solution. Each point represents the mean of triplicate cultures, differing by not more than 10%; the quantity of radioactivity incorporated into cells in parallel cultures not treated with drugs has been subtracted (3,500 c.p.m. for uridine; 600 c.p.m. for thymidine).

Table 1 Effect of cyclic nucleotides and con A on thymidine incorporation.

Additions	Control*	Dibutyryl cyclic AMP, 2×10^{-4}M
None	2.4	3.4
8-Br cyclic GMP, 1×10^{-3} M	13.2	12.3
Con A, 0.5 μg ml^{-1}	89.1	16.2
Con A + 8-Br cyclic GMP	107.3	34.0

Culture conditions and assay are described in the legend to Fig. 1. Thymidine incorporation was measured in triplicate at 44 h. Figures are c.p.m. $\times 10^{-3}$.
*In these experiments dibutyryl cyclic AMP was omitted.

nucleic acid synthesis was assessed at intervals by measuring the incorporation of pulses of radioactive uridine or thymidine into trichloroacetic acid (TCA)-precipitable material.

8-Br cyclic GMP caused time-dependent increases in RNA and DNA synthesis, as indicated by uridine and thymidine incorporation, respectively (Fig. 1). Stimulation by 8-Br cyclic GMP paralleled temporally that produced by con A, although the lectin produced quantitatively greater effects. Uridine incorporation increased early with both 8-Br cyclic GMP and con A, and reached a plateau at about 25 h (Fig. 1*a*). The increase in thymidine incorporation, which was initially detectable at 20 h, plateaued at about 46 h (Fig. 1*b*).

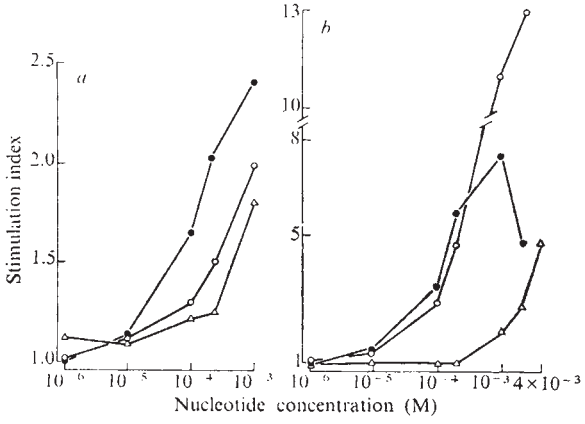

Fig. 2 Stimulation of ^{3}H-uridine (*a*) and ^{3}H-thymidine (*b*) incorporation into TCA-precipitable macromolecules by various concentrations of guanosine 3′,5′-cyclic nucleotides: △, cyclic GMP; ●, dibutyryl cyclic GMP; ○, 8-Br cyclic GMP. Stimulation index (ordinate) is the ratio of incorporation by nucleotide-treated cultures to that by cultures without adding drug (4,100 c.p.m. for uridine and 1,200 c.p.m. for thymidine). Incorporation was measured at 20 h for uridine and 44 h for thymidine. Triplicate points agreed within 10%. The incorporation assay and culture conditions are described in the text and the legend to Fig. 1. Viability of cells (assessed by exclusion of trypan blue) was not affected by the nucleotides at concentrations shown. The spleen leukocytes in these experiments were 90–95% lymphocytes, contaminated with 5–10% macrophages and some erythrocytes. Identical results were, however, obtained if erythrocytes were removed by osmotic lysis and macrophages removed by adsorption to Petri dishes or glass wool.

The stimulatory effects of 8-Br cyclic GMP, dibutyryl cyclic GMP, and cyclic GMP itself, were dose dependent (Fig. 2). At 1×10^{-3} M the cyclic GMP derivatives produced a 2 to 2.5-fold stimulation of uridine incorporation and an 8 to 11-fold increase in thymidine incorporation. Cyclic GMP was less potent and produced mitogenic effects at high concentrations only (4.6-fold stimulation of thymidine incorporation at 4×10^{-3} M). 5′-Guanosine monophosphate did not stimulate thymidine incorporation at concentrations between 10^{-4} and 4×10^{-3} M (results not shown).

Since endogenously synthesised adenosine 3′,5′-monophosphate (cyclic AMP) and exogenous N^6,$O^{2'}$-dibutyryl cyclic AMP (dibutyryl cyclic AMP) have been reported to inhibit the lectin-induced mitogenic activation of lymphocytes[4,5], we asked whether dibutyryl cyclic AMP could inhibit the stimulatory effects of 8-Br cyclic GMP. The dibutyryl cyclic AMP derivative was used at a concentration (2×10^{-4} M) that maximally inhibited con A-stimulated thymidine incorporation without reducing cell viability (measured by exclusion of trypan blue). Dibutyryl cyclic AMP did not affect the stimulation of thymidine incorporation caused by 1×10^{-3} M 8-Br cyclic GMP although it did inhibit stimulation by con A by 80% (Table 1). In addition 8-Br cyclic GMP did not protect con A-stimulated cells from inhibition by dibutyryl cyclic AMP (Table 1). In fact the stimulation of thymidine incorporation by 8-Br cyclic GMP appeared to be approximately additive to that caused by con A (at a concentration found in preliminary experiments to be maximally effective) whether or not dibutyryl cyclic AMP was present. It appears likely that the cyclic GMP derivatives stimulate a subpopulation of splenic lymphocytes smaller than that stimulated by con A.

It has been proposed that cyclic AMP and cyclic GMP act in opposite directions in biological systems, so that the ratio of the two nucleotides within cells regulates many cell functions, including growth[6]. Cyclic AMP inhibits the growth of cells in culture[7], as well as the lectin-induced transformation of lymphocytes[4,5]. We have found that exogenous cyclic GMP and two of its chemical derivatives can stimulate resting mouse spleen lymphocytes to synthesise RNA and DNA, with a time course similar to that caused by con A.

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Do immunoglobulins have proteolytic activity?

As more and more proteins are sequenced, the search for sequential and structural interrelationships among them is becoming a major activity, even though it is still in its infancy compared with investigations dealing with ancestral relationships[1,2]. For our study of homologous regions found among ancestrally unrelated proteins we have modified McLachlan's double matching method[3] and used it to find the statistically significant homologies. The method, the details of which are being published elsewhere[4], enables a computer to perform a sliding match between two proteins, either when both proteins are aligned from N-terminal to C-terminal, which we call 'normal matching', or when one

is aligned from N-terminal to C-terminal while the other is aligned from C-terminal to N-terminal, which we call 'reverse matching'. At each step during this sliding match, some amino acids will be found to be identical between the two proteins being matched and some others will be different. Based on similarities found among ancestrally related proteins the probability of each amino acid being substituted by another is calculated—the *M* score of similarity[3]—and printed. The scores reach from 0, a non-match, all the way to 8 and 9 both of which stand for exact matches, 9 being assigned to phenylalanine, cysteine, tyrosine and tryptophan. For ease of detection, the printout has a stop (.) for a score of 8 and a prime (′) for a score of 9 (ref. 4). The same molecule can also be matched against itself to reveal the presence of repeating subsequences and symmetry patterns[5].

During this study it was observed that the serine-containing active site fragment of bovine trypsin as well as of *S. griseus* proteolytic enzyme matched the variable region of Bence Jones protein λ SH with an exceptionally high *M* score corresponding to a gaeat statistical significance (Tables 1 and 2). More than one homologous region was observed between the histidine, aspartic acid and serine-containing active site fragments of trypsin and Bence Jones λ human SH; in other words there were multiple copies of each trypsin active site fragment with varying degrees of significance, in Bence Jones λ human SH. This suggests to us the possibility for the repeating sequences found in many proteins[6] to be related to a limited number of primordial subsequences from which all proteins could have evolved[5]. We are not aware of any report which links any proteolytic activity with immunoglobulins. The level of significance that was obtained between Bence Jones λ human SH and serine-containing active site fragments of various proteolytic enzymes, however, is so great that we decided to compare the distances between the serine, aspartic acid and histidine active site fragments of trypsin with the distances between various homologues of these fragments on Bence Jones λ human SH.

Table 1 Significant homologies found between the Bence Jones λ human SH and serine-containing active site fragment of various proteolytic enzymes

Target protein	Position number	Matching sequences Individual scores	*M* score	Double matching probability
S. grieseus enzyme	*	TCQGDS ′	49	8.8×10^{-9}
Trypsin, bovine	183	SCQGDS 5′	46	8.8×10^{-8}
Chymotrypsin A & B, bovine	195	SCMGDS 5′ 3 . . .	41	5.7×10^{-6}
Key protein				
Bence Jones λ SH	20	TCQGDS		

Target represents the protein(s) matched and key the protein which is used to find the matching sequences among the target proteins. Position number indicates the position along the protein, of the sequence being matched, numbered contiguously from the amino terminal of the protein. Contiguous numbering is used to avoid confusion arising from many different groups introducing gaps into their sequences for various reasons. The numbers, periods and primes below the target sequences represent the individual similarity scores between the amino acids of the target and the amino acid of the key directly below it. *M* score shows the cumulative score for the span length of the key, which is obtained by summing of the individual scores. Double matching probability gives the probability for such a matching occurring by chance. The f_A used in this matching represents the composition of amino acids of the key[3] and f_B the composition of the universe of proteins given in ref. 1 according to ref. 4.

The single letter amino acid alphabet is used[1], for the sake of indisputable clarity, when alignments are given. S, serine; C, cysteine; Q, glutamine; D, aspartic acid; T, threonine; K, lysine; N, asparagine; A, alanine; F, phenylalanine; R, arginine; M, methionine.

* Not known since only fragments have been sequenced.

Table 2 Significant homologies found between serine-containing active site fragment of trypsin and different Bence Jones proteins

Target protein	Position number	Matching sequences Individual scores	*M* score	Double matching probability
Bence Jones λ human SH	20	TCQGDS 5′	46	8.8×10^{-8}
Bence Jones human HA and NEW	21	SCSGGS . ′ 3 . 3 .	39	4.7×10^{-5}
Bence Jones human VIL and BO	21	SCTGTS . ′ 3 . 3 .	39	4.7×10^{-5}
Bence Jones human X and BAU	20	TCSGDK 5′3 . . 3	36	2.5×10^{-4}
Bence Jones human Kern	20	TCSGDN 5′3 . . 5	38	6.0×10^{-5}
Bence Jones mouse 104E	21	TCRSST 5′5335	30	5.4×10^{-3}
Key protein				
Trypsin	183	SCQGDS		

Legend as Table 1.

A close examination of the three dimensional model of an immunoglobulin made it possible to eliminate many of these possibilities leaving only two regions where the sequences found homologous to the three active site fragments of trypsinogen are reasonably close to each other. One of these regions is located at the amino-terminal end of the light chain (the variable region) completely exposed to the outside. The distances between the three amino acids serine, aspartic acid and histidine are given in Table 3. The other area is in the constant region of the light chain but completely buried inside the molecule (Table 3).

The distances between trypsin active site fragments, calculated from atomic coordinates, is also given in Table 3. Whether any immunoglobulin molecule would exhibit proteolytic activity can only be determined experimentally. We speculate that the area in the variable region of the immunoglobulin exposed to the outside might be the site for proteolytic activity, which would possess very little selectivity. Perhaps a peptide would be needed to bind to this area and bring the distances between the indicated amino acids closer to the distances found between serine, histidine and aspartic acid fragments of trypsin.

Table 3 Distances (in Å) between the active site residues of trypsin and homologous residues of Bence Jones λ human SH

Trypsin	$S_{188}-H_{46} = 8.7$	$S_{188}-D_{90} = 11.0$	$H_{46}-D_{90} = 6.1$
Bence Jones	$P_7-S_{71} = 16$	$P_7-S_{26} = 22$	$S_{26}-S_{71} = 7$
Bence Jones	$H_{176}-S_{198} = 14$	$H_{176}-D_{200} = 18$	$S_{198}-D_{200} = 4$

The distances for trypsin were calculated from its atomic coordinates. The distances for Bence Jones λ human SH were measured from its model. The first set represent the homologous sites at the variable region of the immunoglobulin molecule and the second set the constant regions (see text). Numbers indicate the position of the amino acid contiguously from the N-terminal of the protein.

A recent study[7] has demonstrated that mutants of *Escherichia coli* K12 with deletions of *lac Z* gene can re-acquire the ability to hydrolyse β-galactosides. Full lactose competence was restored through a sequence of at least five mutations. The enzyme evolved differed from the *lac Z* β-galactosidase of the wild type *E. coli* in its immunological, kinetic and sedimentation characteristics. The authors suggested that the adaptation of a gene not involved in lactose utilisation to a form capable of specifying a β-galactosidase is similar to evolution by natural selection.

We believe that the gene(s) coding for the protein whose active site fragment(s) has a three dimensional conformation closest to the active site fragment(s) of the wild type β-galactosidase is the one which underwent five or so mutations to yield the new enzyme[5].

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Specificity of antigen receptors for cytochrome *c* in delayed hypersensitivity

THE specificity of receptors on thymus-derived cells (T) and bone marrow-derived cells (B) for the same antigen is of interest if the various immune responses directed towards protein antigens[1] are to be understood. Detailed molecular studies of such specificities can only be undertaken with protein antigens of known structure. Following a large body of detailed work on the antigenic structure of cytochrome *c* for determinants related to antibodies[2], we report here the beginnings of a similar approach to an analysis of the determinants in cytochrome *c* related to delayed hypersensitivity.

Female albino Hartley guinea pigs (400–450 g) were immunised with 1.0 mg of horse cytochrome *c* in 0.2 ml phosphate buffered saline (PBS) (emulsified with an equal volume of Freund's complete adjuvant (FCA)) by the injection of 0.1 ml into each footpad. Two weeks later the animals were skin tested with 0.1 mg of horse cytochrome *c* in 0.1 ml PBS. The sites of injections were assessed for thickness with a caliper (Schnelltäster, System Kröplin AO2T, Kröplin, Schluchtern, Germany) at 2, 4, and 24 h, as was the diameter of erythema. No significant reactions were noted at 2 or 4 h and injection of unimmunised guinea pigs had no significant effects at any time interval. In addition to the absence of 'immediate' type skin reactions, a search for specific antibody was made by several methods and in every case found to be negative, as has previously been reported[3]. The sera for testing were taken 4 d following the skin test dose in 15 consecutive animals. Passive haemagglutination using the glutaraldehyde method for coupling[4] the cytochrome *c* to sheep erythrocytes was used and titrations were carried out in microtitre plates. All 15 sera had a zero titre while a positive control rabbit antihorse *c* (cytochrome *c*) serum containing 0.1 mg antibody protein ml^{-1} had a titre of 1:160 with such cells. Passive cutaneous anaphylaxis (PCA) was carried out with three serum pools from groups of five animals. 0.1 ml of such pooled sera at a 1/5 dilution gave negative PCA reactions. Finally, all 15 sera were tested by a radioimmunoassay double antibody method[5]. A potent rabbit anti-guinea pig γ globulin serum was used as second antibody. In all 15 sera, 2 μl of immune guinea pig serum bound less than 5% of a tracer dose of 20 ng of ^{131}I-horse cytochrome *c*. We thus concluded that in these animals at the time of testing no detectable circulating antibody was present.

On the other hand, all animals had significant delayed reactivity at 24 h with erythema averaging 1.3 cm (± 0.5) in diameter and in duration averaging 0.65 mm (± 0.3). The specificity of this reaction was then studied by skin testing horse *c* immunised animals with various heterologous cytochromes *c*. The results are illustrated in Fig. 1. Skin thickness relative to the activity of horse *c* is plotted as a function of the number of amino acid differences existing between horse *c* and the heterologous *c* used for skin testing. The specificity of the reaction is quite restricted. Kangaroo, turkey, human, and tuna cytochromes *c* which differ from horse *c* by 7, 11, 12 and 17 amino acids, respectively, give no detectable skin responses in animals immunised with horse *c*. Donkey, bovine, and sheep (the latter two cytochromes *c* have an identical primary sequence) cytochromes which differ from the horse protein by one, three and three amino acids, respectively, are indistinguishable from horse *c* in skin tests while whale *c* (which differs by five residues from horse *c*) is about one half as effective in the skin tests. Dog and rabbit cytochrome *c* which differ by six residues from the horse protein are even less active than the whale protein. As bovine cytochrome *c* exhibits all the skin reactivity of the horse protein, the three sequence differences between them are not the residues conferring specificity in this reaction. The relevant sequence comparisons are listed in Table 1.

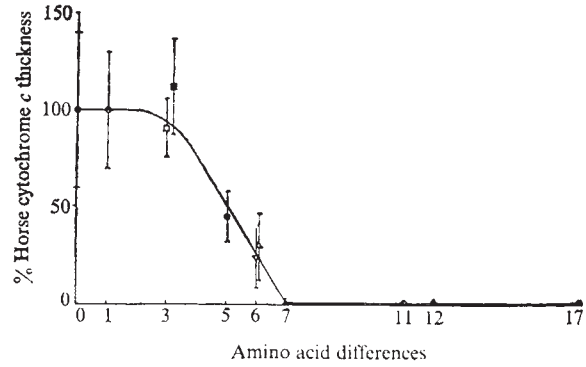

Fig. 1 Cross reactions of various heterologous cytochromes *c* in horse *c* immunised animals. Horse (●), donkey (half filled circle,) bovine (half filled square), sheep (□), whale (diagonal crossed circle), dog (▽), rabbit (△), kangaroo (▲), turkey (diagonal crossed square), human (vertical crossed circle), and tuna (■). The vertical line through the symbol represents one standard deviation of the mean of the skin thicknesses, 24 h after skin test. Sequence data from Dayhoff[16].

The two sequence differences between whale and bovine *c* are important, however, as are the two differences between the whale and rabbit proteins. Sequence position 92 is of special interest since bovine and horse *c* both carry glutamic acid there, while the whale and rabbit proteins carry alanine. In the same region at position 89, rabbit *c* carries aspartic acid while the bovine and whale proteins carry glycine. Thus, there is an order of sequence similarity and differences which parallels the quantitative changes in specificity. In the region encompassing sequence positions 89 and 92, bovine *c* is most like the horse protein, rabbit *c* is the most distantly related and whale *c* is intermediate in structure. There are also amino acid differences at positions 44 and 62 which could contribute to the specificity differences exhibited by the bovine and whale proteins and between the whale and rabbit proteins, respectively.

Table 1 Cytochrome *c* sequence comparisons

	44	62	89	92
Horse	Pro	Glu	Thr	Glu
Bovine	Pro	Glu	Gly	Glu
Whale	Val	Glu	Gly	Ala
Rabbit	Val	Asp	Asp	Ala

Sequence data from Dayhoff[16].

This structural specificity is related to these sequence differences in the context of the three-dimensional structure of cytochrome *c*. The evidence for this is that the cyanogen bromide fragments of cytochrome *c* which comprise the sequences 1–65, 1–80, 66–80, and 81–104 are all inactive in eliciting delayed skin reactions in animals immunised with horse cytochrome *c*. Such data suggest that the determinants responsible for the skin reactivity are very conformation dependent, a situation which is extremely common with antigenic determinants in globular proteins[6,7]. The cross reactions in this delayed-hypersensitivity system are quite limited and, in fact, exhibit a narrower specificity than do the comparable cross reactions carried out with rabbit antibody to horse cytochrome *c* (ref. 2).

This high specificity for the immunogen in cross reactions of delayed hypersensitivity is not restricted to the horse protein. Guinea pigs immunised with either human or turkey cytochromes *c* do not cross react with horse *c* in skin tests. The strength of the homologous reactions is comparable to that of horse *c*-immunised animals and no circulating antibody was demonstrable in either human or turkey *c*-immunised guinea pigs.

Such high specificity has often been demonstrated in delayed hypersensitivity reactions involving the dinitrophenyl-oligolysine system[8] and a number of other systems involving haptenated autologous proteins[9,10]. On the other hand, cross reactions of delayed hypersensitivity to proteins have generally shown a very broad specificity and frequently exhibit good to excellent cross reactions between antigens that do not measurably cross react with specific antibody[11–15]. Thus, the cytochrome system exhibits the type of exquisite specificity more characteristic of delayed reactivity to haptenes than of the type generally characteristic of protein antigens.

The cytochrome system would seem to be an ideal one for the study of the molecular relationship of those structures responsible for interaction with receptors of the two kinds of lymphocytes involved in immune responses.

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Hepatitis B and serum DNA polymerase activities in chimpanzees

THE association of hepatitis B antigen (Australia antigen, HB_s Ag) in human serum with DNA polymerase activity was first reported by Hirschman *et al.*[1]. This has been confirmed and extended by Kaplan *et al.*[2] who found DNA polymerase activity in preparations of concentrated human hepatitis B antigen which were rich in Dane particles. The DNA polymerase activity previously described by Hirschman *et al.*, however, was considered to be dependent on an RNA template, whereas the DNA polymerase reported by Kaplan *et al.* appeared to be a DNA-dependent DNA polymerase.

No studies have been reported on serum levels of DNA polymerase activity in subjects experimentally infected with aetiological agents of hepatitis B. We have infected chimpanzees with hepatitis B viruses[3] to study the temporal relationship between DNA polymerase activity in serum and the appearance of HB_s Ag. Four susceptible chimpanzees were inoculated intravenously with sera containing hepatitis B antigen. Each chimpanzee was monitored for serum HB_s Ag and corresponding antibody (anti-HB_s) during the course of infection by a solid-phase direct radioimmunoassay (RIA) system[4]. Liver damage was assessed by measuring serum glutamic pyruvate transaminase (SGPT) activity and by histological examination of liver biopsy tissue. Serum samples were obtained under anaesthesia three times weekly, and assayed for DNA polymerase and HB_s Ag or anti-HB_s. For DNA polymerase we used a modification of the method of Robinson and Robinson[5].

Of four animals inoculated, three developed infections characterised by the occurrence of transient HB_s Ag followed by anti-HB_s production. In all three hepatitis B was confirmed enzymatically and histologically. The fourth animal did not develop HB_s Ag or evidence of hepatitis but produced late-occurring anti-HB_s 70 d after inoculation.

In all four animals we found spikes of DNA polymerase activity, the first increases being uniformly observed between the 20th and 40th days after inoculation. In the three animals who experienced antigenaemia and hepatitis, first spikes in DNA polymerase either closely preceded or paralleled the first appearance of HB_s Ag and always occurred before first increases in transaminase levels. Figure 1 shows these relationships for one of the three animals (Ike), and reveals a second DNA polymerase peak post-dating the bulk of the antigenaemia and closely preceding the transaminase peak. In the other two animals experiencing hepatitis, increases in DNA polymerase activity clearly preceded the first appearance of HB_s Ag (by 20 d in one animal, and by 25 d in the other) and similar secondary

Table 1 Effect of reaction conditions on DNA polymerase activity (Ike) in two serum pools

	Complete, no additions	−NTPs	+Act D	+DNase	+RNase	+poly (dA-dT) poly (dA-dT)
Day 26 Pool	129*	36	23	79	121	77
Day 33 Pool	411	137	50	128	405	304

*Net c.p.m. (gross c.p.m. less background and zero-time c.p.m.).
Pools of serum obtained on days corresponding to DNA polymerase peaks occurring on days 26 and 33 were tested for the effects of various additions and deletions on the incorporation of ^{3}H-dTTP into a TCA-insoluble product. Final reaction-mixture concentrations of the additions were: actinomycin D, 10 μg ml^{-1}; DNase (EC 3.1.4.5), 10 μg ml^{-1}, 30 min at 37° C; RNase (EC 2.7.7.16), 10 μg ml^{-1}, 30 min at 37° C; poly(dA-dT)·poly(dA-dT), 25 μg ml^{-1}.

polymerase peaks appeared in both and coincided with increases in transaminase levels.

As Fig. 2 shows, the serum of the fourth animal (Jay) showed spikes in DNA polymerase activity between the 20th and 40th days and the 50th and 70th days after inoculation in the absence of detectable HB_s Ag or increases in transaminase activity. Anti-HB_s was first detectable on day 70.

Characterisation of the two DNA polymerase activities found in Ike's serum is summarised in Table 1. Both were inhibited by actinomycin D (10 μg ml^{-1}) or poly(dA-dT)·poly-(dA-dT) (25 μg ml^{-1}), indicating that these activities do not depend on an RNA template. Preincubation of the day 26 pool and the day 33 pool reaction mixtures (Table 1) with ribonuclease did not affect the incorporation of ^{3}H-TMP into trichloroacetic acid (TCA)-precipitable products, further suggesting that these polymerase activities do not depend on an endogenous RNA template. The products of the polymerase reactions, however, were partially sensitive to DNase. Omission of nucleoside triphosphates from

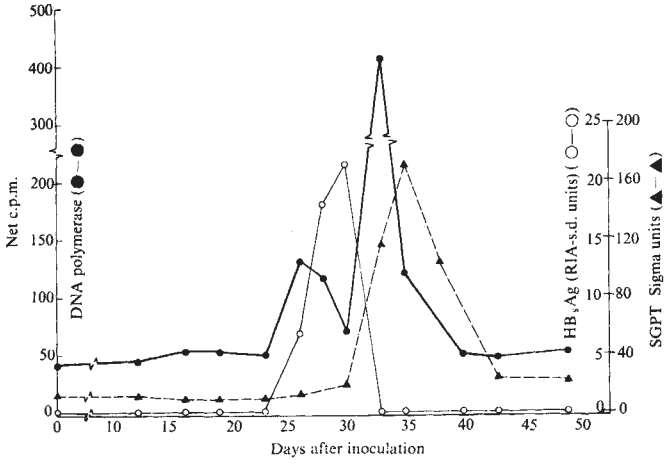

Fig. 1 Chimpanzee Ike. DNA polymerase activity in serum was determined by a radioisotopic method which measures the incorporation of ^{3}H-dTTP into TCA-insoluble product. The polymerase assay contained in a volume of 0.300 ml: 50 μl serum; 200 μl 0.1 M Tris-HCl buffer, *p*H 8.3; the balance of the reaction mixture contained (final assay concentrations) mercaptoethanol (2.5 mM), dATP, dCTP, dGTP (0.25 mM each), $MgCl_2.6H_2O$ (5 mM, unless otherwise noted), KCl (40 mM), and ^{3}H-dTTP (0.93 μM). The reaction mixture was incubated for 60 min at 37° C; 100 μl of the mixture was pipetted into 5 ml of ice-cold 5% TCA+2% pyrophosphate solution to precipitate the labelled product. The precipitate was collected on a Millipore filter (EAWPO2500) and washed repeatedly with 5% TCA+2% pyrophosphate, 80% ethanol and dried with diethyl ether. The disk was placed in 10 ml of toluene PPO-POPOP cocktail for counting. Background and zero-time assays c.p.m. were subtracted to yield net c.p.m. Serum glutamic pyruvate transaminase (EC 2.6.1.2) was measured by colorimetric Sigma procedure. Serum HB_s Ag was determined by a solid-phase direct radioimmunoassay (AusRIA, Abbott Laboratories). Results for HB_s Ag are expressed in standard deviation (s.d.) units. Preinoculation chimpanzee serum polymerase activity averaged 24 c.p.m.

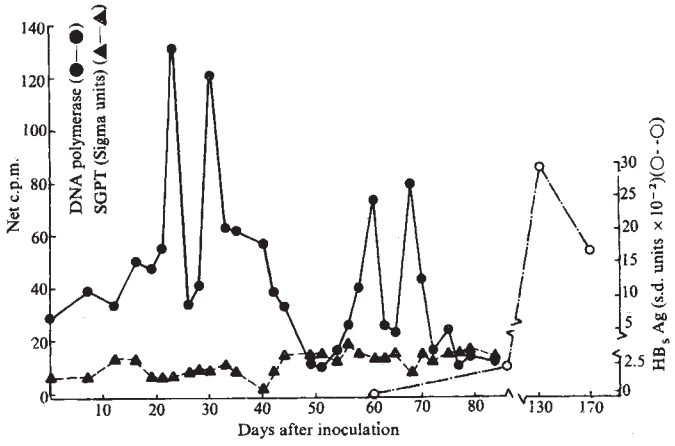

Fig. 2 Chimpanzee Jay. Serum assays are identical to those in Fig. 1, except that 15 mM Mg^{2+} was substituted for 5 mM Mg^{+2}; the final assay was 0.1% in Triton X-100.

either of the reaction mixtures resulted in decreased incorporation of ^{3}H-dTTP into acid insoluble products.

Based on the results of our study of DNA polymerase activity in four chimpanzees infected with hepatitis B viruses, we believe two preliminary conclusions can be drawn. First, for animals experiencing hepatitis, DNA polymerase activity appears in the serum before the onset of antigenaemia and liver damage and may represent replication of viral DNA. Second, the postulated viral DNA polymerase activity (in Ike) seems to depend on a DNA template rather than on an RNA template—this idea is consistent with the findings of Kaplan *et al.*, who demonstrated that DNA polymerase activity associated with the Dane particle was inhibited by actinomycin D and daunomycin. Furthermore, the detection of increased DNA polymerase activity in an infected animal (Jay) who showed no clinical, enzymatic (SGPT) or immunological (HB_s Ag) evidence of hepatitis during the first 70 d after inoculation suggests the DNA polymerase assay may be useful in screening potentially infective sera.

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Release of enzymes from cell walls by an endopectate-*trans*-eliminase

THE cell walls of higher plants were long regarded as essentially inert structures which functioned mainly as mechanical barriers to the expansion of protoplasts under turgor pressure and as skeletal supports in tissues. It is now known, however, that they contain a number of hydrolytic enzymes at least one of which is a hydroxyproline-rich protein[1]. These enzymes are often of types associated with the dynamic recycling functions of intracellular lysosomes[2-4]. They have usually been detected by the activity of cell wall preparations after comminution of tissues and removal of contaminating cytoplasm by washing[1,2,5] or by the activity of intact or excised roots[3,6]. We now report on the release of wall bound enzymes from living cells by a pectic enzyme.

An endopectate-*trans*-eliminase (PTE) was obtained from 3-d-old soft rots of cucumbers caused by the bacterium *Erwinia carotovora* (NCTC 1747). One volume of rot was extracted with one volume of 0.85% NaCl and made cell-free by centrifuging. Ammonium sulphate was added to 61–80% saturation, the precipitate collected, dissolved in, then dialysed against water, and fractionated by elution from Sephadex G75 superfine with 0.1 M NaCl in 0.005 M Tris buffer, *p*H 9.0. Fractions containing most of the PTE activity were subjected to isoelectric focusing between *p*H 7.0 and 10.0 for 52 h at 4° C. Fractions containing PTE were combined, dialysed and gave a single band after electrophoresis on polyacrylamide gel. The preparation did not degrade any of a wide range of cell wall and other polymers other then those based largely on anhydrogalacturonic acid residues. One unit of activity of this PTE gave an increase in absorbance at 550 nm of 0.1 in 30 min at 25° C in the following assay: 1 ml enzyme solution, 2.5 ml 1% sodium polypectate (Sunkist Growers Inc.), 0.5 ml $CaCl_2$ (final 0.001 M), Tris buffer, *p*H 9.0 (final 0.1 M), left at 25° C. Then 7.5 ml of a mixture of 5 ml thiobarbituric acid (0.04M) and 2.5 ml HCl (1M) was added and the red colour produced estimated at 550 nm against a reagent blank.

Table 1 Effect of endopectate-*trans*-eliminase (PTE) on release of K^+ and enzymes from potato tuber tissue

Activity†	Treatment*					
	a	*b*	*c*	*d*	*e*	*d*+*e*
K^+	625	17	685	715	60	775
Acid phosphatase	0.132	0.059	0.056	0.040	0.121	0.161
Phenoloxidase	0.13	0.032	0.059	0.046	0.12	0.166
Peroxidase	0.10	0.060	0.023	0.008	0.083	0.091
Malic dehydrogenase	0.132	0.082	0.115	0.052	0.22	0.272

Values shown are corrected for slight changes caused by autoclaved enzyme.

* *a*, 3 ml water containing 14.8 units PTE, 0.001 M $CaCl_2$, 0.02 M Tris buffer (*p*H 9.0) for 3 h at 22° C; *b*, as *a* but in 0.5 M sucrose as plasmolyticum; *c*, as *b*, then liquid removed, disks washed in 0.5 M sucrose, added 3.0 ml water for 10 min; *d*, 3 ml, frozen at −20° C, thawed and kept for 10 min; *e*, liquid removed, disks then treated as *a*.

† K^+ activity by flame photometry (p.p.m.). Acid phosphatase by estimating *p*-nitrophenol liberated from *p*-nitrophenyl phosphate at 400 nm (ref. 7); activity was expressed as mg *p*-nitrophenol liberated per 0.2 ml enzyme solution. Phenoloxidase by estimating oxidation of catechol[8]; activity was expressed as ΔOD 495 nm min^{-1} per 0.2 ml enzyme solution. Peroxidase by estimating oxidation of pyrogallol in the presence of H_2O_2 (ref. 9); activity was expressed as ΔOD 420 nm min^{-1} per 0.01 ml enzyme solution. Malic dehydrogenase by estimating oxidation of NADH in the presence of oxalacetic acid[10]; activity was expressed as ΔOD 340 nm min^{-1} per 0.1 ml enzyme solution.

Samples of 48 disks (0.5 × 7.0 mm diameter) from medulla of turgid potato tubers (cv. Majestic) were treated in various ways.

At the end of each treatment, the ambient liquid was collected and assayed for K^+, acid phosphatase, phenoloxidase, peroxidase and malic dehydrogenase.

The 3 h treatment of tissues with PTE caused cell separation and death of protoplasts in the sense that they did not plasmolyse in hypertonic solutions; treatment with enzyme in a plasmolyticum still caused cell separation but protoplasts remained plasmolysed and intact.

Table 1 summarises the effects of the different treatments and also shows in column *d* + *e* the total of K^+ and enzymes liberated from frozen tissue after thawing and released from residual tissue after treatment with PTE for 3 h. Comparison of columns *d* and *e* show that K^+ liberated by PTE treatment of residual tissue was only about 10% of that liberated by freezing followed by thawing. The corresponding figures for the enzymes were 72–91%.

Similarly, although very little K^+ was released from tissues treated with PTE in a plasmolyticum, large amounts of peroxidase, acid phosphatase and malic dehydrogenase and smaller though substantial amounts of phenoloxidase were liberated in these conditions. There were corresponding differences when plasmolysed protoplasts in PTE treated tissue were rapidly deplasmolysed.

Because PTE is believed to act only on substrates in cell walls and middle lamellae, the results suggest that large proportions of the four enzymes in cells are bound to cell walls and are liberated when these are exposed to PTE. This confirms earlier work which showed that cell walls contain substantial amounts of peroxidase[1] and acid phosphatase[5] but is unexpected for malic dehydrogenase (which is known to occur as different isoenzymes in mitochondria, chloroplasts and cytoplasm[11] but not, so far as we know, outside the plasmalemma).

We have considered the possibility that our results could be explained, at least in part, by adsorption by cell walls of cytoplasmic enzymes released when cells are broken during preparation of the potato tuber disks or by residual cytoplasm on cut surfaces. But this seems unlikely because preliminary washing of disks in 0.2 M NaCl did not decrease release of acid phosphatase or malic dehydrogenase when disks were later treated with PTE in a plasmolyticum. It seems, therefore, that these four enzymes are released from cell walls by the action of PTE which is highly specific and is believed to be confined to the rupture of α–1,4–D–glycosiduronic linkages. This poses the question of the relationship between polymers based on these linkages and wall-bound protein and, of course, the function of wall-bound enzymes particularly such as malic dehydrogenase hitherto regarded as essentially cytoplasmic.

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reviews

Simple guide to welfare economics

In Defence of Economic Growth. By Wilfred Beckerman. Pp. 287. (Cape: London, June 1974.) £3.95.

THIS book is precisely what it claims to be: a defence of economic growth. It is written with wit and verve, in a forensic style which would do credit to a barrister defending a criminal before a jury. The prosecution witness (the mass media, fuddy-duddy scientists, young radicals, and the middle classes) are battered by facts, arguments and ridicule. It is, as Beckerman frankly warns his readers, a one sided contribution to a debate. The issue is familiar enough. Should economic growth be curtailed or even phased out as a deliberate social policy, in order to prevent pollution and to conserve resources for future generations? And, to anyone interested in this issue, Beckerman's views are familiar enough too. Market forces, either working naturally or modulated by a system of taxation, will continue, as they do at present, to regulate the level of pollution and the use of resources. Zero growth would exacerbate the inequalities between rich and poor; it would be of dubious benefit to future generations, and in any case it would be politically impossible to achieve in a democratic society.

Many people, including some scientists, do not realise that the economist, dealing with the theory of choice in the allocation of resources, is (or tries to be) as neutral over value judgements as the chemist is when he deals with the theory of valency. It is not the economist's professional business to moralise about how the national product should be allocated among such items as present consumption, investment in the future, and conservation of the environment. His business is to say what the economic consequences will be for different patterns of allocation. Thus in the mind of the economist pollution is not 'good' or 'bad' any more than arsenic is 'good' or 'bad' in the mind of a chemist. The notion that conservationists have to defend the environment against hordes of economists, hell-bent on maximising economic growth, is, as Beckerman convincingly argues, nonsense. The economist is interested in the logic, not the ethics, of choice.

A clear distinction has to be made between two dimensions of choice: one is how to allocate resources at any moment of time (for example, as between public and private transport); the other is how to allocate resources over a period of time (for example, as between 1975 and 1995). The controversy over economic growth lies in the second of these two dimensions. It is, crudely, the choice between on the one hand, more consumption now and possibly (though Beckerman disputes this) less for our grandchildren: and, on the other hand, some sacrifice of present consumption and (again only possibly) more for our grandchildren.

The evidence of history is that improvement in economic welfare has been accompanied by improvement in social welfare. As nations have become more affluent their citizens have, on the whole, become more healthy, enjoyed greater security, shared more fully those immaterial benefits we associate with 'quality of life'. So, argues, Beckerman, continued economic growth is in the best interests both of today's poor and of posterity. As for the social costs of economic growth, for example pollution and disfigurement of the landscape, the economists have an elegant formula as a guide to policy. Pollution (which Beckerman selects to exemplify his views) should not be minimised: it should be optimised at the level above which the marginal cost of more abatement would exceed the marginal cost of the damage done if it were not abated. And to ensure that the cost of disposing wastes into the environment is internalised, just as the cost of labour is, Beckerman proposes a hit-or-miss taxation system which would aim at optimising pollution.

The trouble about the economists' elegant formula is that they cannot quantify it in a way which would command the confidence of anyone accustomed to the rigours of scientific method. The costs of abating pollution can be measured. But estimates of the damage done by pollution which try to quantify the disamenity of fogs and dirty rivers and smells are (to my mind) totally unconvincing. Beckerman himself emphasises that economic welfare is only part of social welfare. The task of politicians is to promote the whole of social welfare. The issue which his book raises is a fundamental one. Is social welfare so conditioned by economic welfare that politicians can exclude the unquantifiable components of social welfare? Or—and this is Beckerman's view—should one go on trying to quantify the whole of social welfare, in money terms because money is a convenient symbol of value? After all, he says, it was a long time before physicists learnt how to measure heat. Or are the unquantifiable components in the cost-benefit equations of social welfare so important that this whole quasi-scientific approach to social policy ought, for the time being, to be regarded as under suspicion?

Beckerman, quite rightly, withers with criticism the specious precision of computer simulations about the prospects for mankind. But is he not himself exposed to a somewhat similar, though much milder, criticism? There is growing evidence that social welfare includes much more than economic welfare and that in affluent societies the correlation between the two (let alone causation) cannot be assumed to be strong. Much of what social welfare means cannot be quantified. Is there not a risk that the value-free approach to welfare economics will over estimate the quantifiable components and under estimate the others and, in doing so, distort the issues instead of clarifying them?

One puts this challenging book down with the impression that economists, just because they are liable to become involved in social policy, cannot remain as disengaged from value judgements as scientists can. The scientist can exclude from consideration phenomena he cannot quantify. He would discard an equation for which he could not supply data. The economist analysing social choice is tempted to go on using the equation even though he cannot supply the data. Beckerman has not altogether resisted this temptation.

E. ASHBY

Physics and Chemistry from Longman

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An account, drawn from several different branches of physics, of the physical aspects of X-ray production. "The book is economically written, well illustrated and carefully produced. It should be made available in all scientific libraries, and should find a place on the shelves of any individual who is interested in the production, properties and applications of short wave-length radiation"
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Radiation Chemistry: An Introduction
A J Swallow
Intended for final year undergraduate students of chemistry, for those taking graduate courses in the appropriate subjects, for those beginning research in radiation chemistry, and for specialists in related fields who wish to obtain a general knowledge of the subject. A general knowledge of chemistry and especially chemical kinetics is assumed, at approximately second year B.Sc. level. £5.50 net

Stereochemistry
G Natta and M Farina
This book starts with a review of stereochemistry from Pasteur to the present day and follows by describing the shapes of molecules, molecular symmetry and the dynamic aspects of reactions. It then continues with a consideration of macromolecular stereochemistry, biological macromolecules and the structure of chemical compounds. It closes with a review of optical activity. £2.40 net

Tables of Physical and Chemical Constants
G W C Kaye and T H Laby
A classic reference work with extensive tables of conversion factors from older systems into SI units. Parts of the book have been completely rewritten, while others have been revised. New sections have been included. £3.95 net

Longman 1724-1974

Silent synapses

Memory and Nerve Cell Connections. By Richard Mark. Pp. x+15. (Criticisms and Contributions from Developmental Neurophysiology.) (Clarendon: Oxford; Oxford University: London, June 1974.) £2.95.

In this very interesting book Richard Mark (well known for his work on selective neuromuscular reinnervation in fish and newts, in which he has shown that, in certain circumstances, neuromuscular synapses in lower vertebrates may become functionally switched off without apparent morphological change) extends his arguments on "silent synapses" to the central nervous system and attempts to provide thereby a skeletal framework for a theory of memory and learning. The book is well organised, well written and should be read.

The nature of the audience at which the book is aimed is not clear, since though the introductory outlines of the structure and function of neurones, glia, synapses, brain codes and so forth are somewhat elementary, the arguments introduced later are fairly sophisticated. This does not really matter, however, and students and research workers alike will find this book, which is organised rather like a detective story, highly readable. After a brief survey of some relevant aspects of neurophysiology the author takes the reader on a tour of various possible mechanisms of memory (with their attendant disadvantages) and finally, eight pages before the end of the book, Dr Mark's own hypothesis, the component parts of which had previously been introduced, is put together. The hypothesis essentially suggests that, in the highly convergent (and initially redundant) neural network of the central nervous system, the less used pathways may become functionally suppressed. Used pathways may become more efficient and this increased usage is held to be associated with increased synthesis of specific marker proteins, leading to long term changes in functional connectivity.

The general argument presented by Dr Mark is interesting and plausible. He makes the good point that "it is not reasonable to abstract the verbal or logical representation of each component of a particular behavioural situation that can be used to demonstrate learning and then demand a physiological explanation for all these abstractions at the same biological level. Yet many physiologically inclined workers have simply taken each of the parameters of a conventionally accepted learning paradigm such as classical conditioning and assumed that there is a cellular explanation for each of them". The point being made here is that the phenomena of learning and memory are highly complex and that simplistic attempts to identify individual structures with these functions are unlikely to be fruitful. From a somewhat similar viewpoint Dr Mark briefly reviews the popular work on memory transfer ('memory molecules') and comes to the tentative conclusion that, although some of the experimental observations may be valid, the hypothetical frameworks associated with this sort of work have so far been inadequate. "It would be imprudent to deny that chemically transmissable behaviour exists. One has the uneasy feeling from all this literature, contradictory and incomplete as it is, that something is there."

Dr Mark makes the interesting criticism that, since the result of memory transfer experiments, even if valid, cannot be fitted into the present framework of biological understanding, the lack of coherence of these experiments with the other brain studies may rule out scientific progress along these lines. "The most telling criticism of chemical memory transfer experiments is not to deny the possibility of the effects, or to denigrate the investigators, but to say that they are before their time. This is just as serious a scientific criticism as to say the work is old-fashioned and repetitive. It means that even if research stumbled upon something of real significance, which perhaps it already has . . . the finding could not be exploited because of the surrounding chasms of ignorance as to other aspects of brain function".

Taking his example from the study of nerve regeneration in amphibians and fishes, Mark instances work on plasticity of synaptic connections which seems to be due to a competitive suppression of transmission in some terminals. In these cases the final pattern emerges not because the best fitted connections improve but because the inappropriate are eliminated. It is proposed that a mechanism akin to this developmental shaping of the nervous system may continue into adult life and work there to modify brain connections into a more appropriate form for current experience; that is, as a basis for learning.

The details provided by Dr Mark to support this idea are, naturally somewhat scanty; and it may well turn out that the whole approach is misleading. It is certainly true, however, that the existence of "silent synapses" peripherally, taken along with the recent evidence for functionally induced plasticity of central connections in the visual system, provokes very interesting speculations. This may not be the most direct route to the mysteries of memory and learning, but the search for silent synapses in the CNS is now on.

R. M. Gaze

Materials in use

Metals, Ceramics and Polymers: An Introduction to the Structure and Properties of Engineering Materials. By Oliver H. Wyatt and David Dew-Hughes. Pp. xiv+640. (Cambridge University: London, May 1974) £12.00 boards; £4.95 paper.

OF books on Shakespeare's plays, the Malaise of Western Man, and materials science, there is no end. No blinding new insights are to be expected on any of these topics: selection, clarity and economy must be our touchstones. Anyone with a shelf-full of such books must be tempted to pluck out some of them and follow Prospero's heroic resolution: "And deeper than did ever plummet sound I'll drown my book".

The volume under review is not for drowning. It is one of the very best of the many English-language texts on materials that have appeared in recent years. Unlike some of these, the new book has a pronounced engineering bias: the fact that Dr Wyatt worked for years in an engineering school which prepares its students for what used to be called a Mechanical Sciences Tripos shows in the special emphasis on mechanical behaviour which is evident in many parts of the book. Thus, although there is a sound summary of the present understanding of dislocation behaviour, macroscopic plasticity theory gets exceptionally thorough treatment; the application of this theory to metal-working processes is unusual in a materials textbook. Ceramics and polymers each receive a good concise presentation, including for the former an informative account of the setting of concrete: glasses are rather too much compressed (a state possibly natural to the material). There is an excellent chapter on steels, particularly thorough on the problem of heat-treating objects of various sizes. The electrical and magnetic chapters are also well presented, with more emphasis than normal on superconductivity (one of Dr Dew-Hughes's special interests) and dielectric materials. Magnetic materials place much more emphasis on domain behaviour than intrinsic magnetism—a good test of fitness for engineering purposes.

The treatment of particular materials is underpinned by a fairly conventional introduction to topics such as crystallography, diffusion, phase diagrams (including a good treatment of ternaries) and band theory. As so often, the thermodynamic treatment is too concise for real clarity; in particular, the statistical basis for entropy has to be taken on trust, which must tinge the crucial concept of an equilibrium population of vacant lattice sites with a touch of black magic. But on the whole, the theoretical underpinning is very clear and sufficiently comprehensive.

It is easy enough to pick out individual small inadequacies and as always these largely reflect the reviewer's predilections: there is almost nothing on the important subject of recrystallisation; the intriguing explanation of how doping with an impurity permits the removal of pores in sintered alumina is muffed; the crucial importance of crystalline states in polymers receives only the most glancing recognition. But the list of these shortcomings (which another reviewer might deem of little consequence) is short and the positive achievement of balance and clarity is very considerable.

Each chapter has a collection of questions, partly numerical and partly purely verbal, designed to test conceptual understanding, and each has an excellent bibliography.

As a basis for a comprehensive course on materials for engineering students, or as a foundation for the much smaller tribe of material specialists, this book can be warmly recommended. The paperback version in particular is excellent value.

R. W. CAHN

Valence calculations and molecular orbital theories

Ab Initio Valence Calculations in Chemistry. By D. B. Cook. Pp. ix+361. (Butterworth: London, March 1974.) £7.50.

THIS is a book which describes the technical aspects of computing wave functions and energies of electrons in molecules as approximate solutions of the Schrodinger equation. It is concerned only with the *ab-initio* method, that is, the approximations contained in such calculations arise from the termination of series expansions of the wave functions rather than the use of a model Hamiltonian. In addition to the technical aspects of the book the author gives a personal justification for the place of such calculations in chemistry which may be summarised as follows. Many of the important concepts of valence theory are not observables, but in order to maintain contact with and influence the main stream of chemical thinking, quantum chemistry must apply itself to the analysis, criticism and quantitative investigation of these concepts. In other words the book is not written just for chemists who want numbers, but also for those interested in ideas.

The book fills a gap in the current literature. It does not set out to present a broad view of quantum theory or valence theory and therefore assumes a fair amount of basic knowledge in these areas. It is elegantly written and printed and I believe it will be widely accepted as a very useful book.

Molecular Orbital Methods in Organic Chemistry—HMO and PMO: An Introduction. By William B. Smith. Pp. xi+161. (Studies in Organic Chemistry, vol. 2.) (Dekker: New York, April 1974.) $14.50. £6.00.

IT is thirteen years since the first books on molecular orbital theories in organic chemistry appeared and one must admire the courage of both author and publisher in adding another volume to this crowded market. About half the book is devoted to an elementary account of Huckel theory (HMO) and its perturbative approximations (PMO). The remainder gives a wide coverage of organic reactivity including the recently popularised symmetry rules. As an elementary account of the subject it is quite acceptable but it is hardly value for money when compared with other books that are available.

J. N. MURRELL

1975 Catalogue of

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may be obtained from

Collet's

Denington Estate
Wellingborough, UK

CHAPMAN & HALL
11 New Fetter Lane,
London, EC4P 4EE.

Buffers for pH and Metal Ion Control
D. D. PERRIN and BOYD DEMPSEY.
September 1974: 184 pages: illustrated: hardback: 412 11700 2: £3.50

Practical Manuals Series
This is a practical book designed to meet the varied needs of those scientists who require pH or metal ion buffers having specified values. Information has been gathered together into numerous pH buffer tables, and these include details of zwitterion buffers. Through the use of worked examples the reader is enabled to calculate the composition of buffers for particular conditions, and small computer programmes are included which facilitate these calculations.

Second Edition
Practical Inorganic Chemistry
Preparations, Reactions and Instrumental Methods
G. PASS and H. SUTCLIFFE
Second edition: September 1974: 256 pages: illustrated: 412 12690 7: hardback: £2.60.

This second edition of a successful textbook is now in SI units and new experiments involving ion-exchange and solid-liquid chromatography have been added.
The experiments described are complemented by suitable structural and analytical studies with follow-up exercises which require the study of listed references.

Second Edition
The Spectroscopy of Flames
A. G. GAYDON, F.R.S.
Second edition: September 1974: 420 pages: illustrated: hardback: 412 12870 5: £8.00.

This book deals primarily with the use of spectroscopic observations to interpret combustion processes in flames and flame structure. It represents a total revision of the author's earlier work so that it now incorporates the latest refinements in techniques as well as some important related advances.

Further information on these titles and a list of stockists is available from the publishers on request.

Impact of climate

Climatic Resources and Economic Activity: A Symposium. Edited by James A. Taylor. Pp 264. (David and Charles: Newton Abbot and London, June 1974.) £6.50.

THIS volume is the latest in a series emanating from the Aberystwyth Symposia, hardy annual gatherings from 1958 to 1973. These have focused attention usefully on what is broadly termed "applied climatology". Inevitably this volume overlaps the contents of certain of its predecessors in such an amorphous field, notably *Weather and Agriculture* (Oxford, 1967), *Weather Economics* (Oxford, 1970), and *Weather Forecasting for Agriculture and Industry* (David and Charles, 1972).

It claimed in the preface that this book is timely in reviewing relationships between climatic resources and some of their social and economic usages: this is indisputable, although the range of papers it contains is broad rather than all-embracing, and the depth is distinctly moderate. Many workers may take greater exception to the unqualified assertion that the volume is "representative of current research trends and achievements" in environmental science. There are other kinds of trends and achievements too, several arguably of greater significance in the long run. Perhaps the most unnecessary aspect of the introductory keynote is its attempt to justify this collection of articles in terms of current geographical philosophy. One wonders whether the three-quarters of the contributors who are employed outside academic geography welcome being seen in this perspective. If applied climatology and meteorology are fields worthy of investigation, they may be explored best if the directions from which they are approached are totally unprejudiced.

The contents include a broad introduction to the concept of the atmosphere as a global resource, and five chapters of bioclimatic interest, almost wholly concerned with weather, plants, and crop husbandry. Two further chapters—more helpful and interesting than most—appraise water resources in the United Kingdom, and mathematical models designed to facilitate the planning of their exploitation. Two chapters follow on air pollution, then a rare, thought-provoking essay on weather and road accidents. The two last contributions are concerned with modelling influences of atmospheric behaviour on general and national economical activities.

Several chapters suffer from the current abuse of statistical methods for environmental analysis. Correlations are based on tiny populations, and rakish linear regression lines are employed where curvilinear relationships even of only quadratic or cubic forms would have yielded much more significant values of r^2. Why weaken a potentially important argument if the chosen "best fit" statistical statement does it less—even much less—than justice?

As an ardent disciple of the school which prefers its scientific books to be more akin to disposable paper cups than presentation clocks, I am bound to think that the circulation of this book will be restricted by its style and cost. It is too lightweight and piecemeal to serve as a worthy reference volume on the library shelves; meanwhile, it is too wasteful in production and design to recommend itself to the college student or the weather-conscious layman. Presumably, therefore, it is aimed at the business market, where its value will depend on the nature of the enterprise. A paperback version could have been recommended to students.

Both front and back cover pictures are from the 1960s, which perhaps might just be forgiven. But the satellite photograph of South America on the back cover would look considerably better—and make more sense to the amateur—if it were not upside down.

E. C. BARRETT

Climate and Life. By M. I. Budyko. English edition edited by D. H. Miller. Pp. xvii+507. (International Geophysics Series, vol. 18.) (Academic: New York and London, April 1974.) $35; £16.45.

THIS book provides the most complete account in English of the climatology work at the Voeikov Geophysical Observatory, and with very complete references at the end of each chapter it is important simply as a reference guide to this work in the Soviet Union.

Apart from this, it is a book which will be of real value to the serious student of climatology, although it is certainly not always easy to read. Starting from solar radiation and heat balance at the Earth, the study of climate and its impact on life is considered within a unified framework which builds upon the work reported in the earlier (1956) summary of research at Voeikov Observatory.

I found the sections on climatic change particularly interesting and useful; I would have preferred to find less emphasis on the effects of volcanic dust, perhaps—or at least comparable emphasis on other factors—and I would take issue with the statement that "over a duration of several centuries, noticeable fluctuations of climate can occur only in high latitudes". But these are relatively minor points and in a book covering so much it is no surprise if the author fails to please all of the readers all of the time.

JOHN GRIBBIN

Earth science

Geology, Resources and Society: An Introduction to Earth Science. By H. W. Menard. Pp. xi+621. (A Series of Books in Geology.) (Freeman: San Francisco and Reading, May 1974.) £6.10.

FROM the days of Lyell to those of Arthur Holmes and Jim Gilluly students of geology have been fortunate in the number of outstanding geologists who have taken on the task of preparing textbooks on their subject. To a remarkable degree a few textbooks of the first rank have contributed to the way geology has evolved, setting a pattern of thought which was to persist for several decades.

Menard states in his preface that he is aiming this book at students who are not majoring in the earth sciences. In other words this is a professional's textbook aimed at a wider audience and one which, in my opinion, may well become a guideline for those who are attempting to integrate various branches of the earth sciences using the term in its widest sense.

Menard's approach is to provide readable syntheses on four or five fundamental divisions of each science, giving himself enough elbow room to treat the essentials but not so much as to become involved in detail. In succession he takes up the subjects of the planet Earth, its deformation, the atmosphere and hydrosphere, and the manner in which these react with the solid rock, first to render it unstable and to weather it and then to produce the sediments. Along the way he includes a brief section on volcanism and concludes with some 80 pages on water, fuels and mineral resources. He follows Holmes rather than Lyell in treating the Earth as a machine and making this, not the stratigraphical record, the link-pin of his arguments. I think he is particularly successful in conveying a sense of proportion and of the relative importance of phenomena. When discussing the circulation of water, for example, he points out what a minute fraction is to be found at any one time in rivers and in the atmosphere; only 0.001% of all the Earth's water is in the air. Menard describes the circulation of solid rock, of sediments, air and water as we understand these matters today. His text would provide an excellent basis for any course dealing with the physical environment.

The title indicates Menard's wider interests. These emerge repeatedly through the book, but apart from the chapter on resources amounting to less than 10% of the whole, and the section on engineering problems caused by unstable ground, no extended portion of the text is devoted to them, though Menard does his best to emphasise their importance in the approach he adopts throughout the volume. It is difficult to see how he could have done more. He has been remarkably successful in his syntheses which in my mind constitute the main virtue of this book.

J. SUTTON

obituary

Alfred E. Mirsky

PROFESSOR ALFRED E. MIRSKY, who first isolated and characterised genetic material from mammalian cells, died on June 19, 1974. He was 73.

Born in New York, he gained his PhD from the University of Cambridge in 1926 and returned to the Rockefeller University in 1927. He became a professor there in 1954 and retired in 1971.

Sir Frederick Brundrett

SIR FREDERICK BRUNDRETT, KCB, KBE, former Scientific Adviser to the Minister of Defence, died on August 1, 1974. He was 79.

After attending the University of Cambridge, he joined the wireless branch of the Royal Naval Volunteer Reserve in 1916. He became Chief of the Royal Naval Scientific Service in 1946, and was appointed Deputy Scientific Adviser to the Minister of Defence in 1950. In 1954 he became Scientific Adviser and Chairman of the Defence Research Policy Committee.

Gertrude E. Perlmann

PROFESSOR GERTRUDE E. PERLMANN, an authority on protein chemistry, died on September 9, 1974. She was 62.

After receiving her DSc, from the German University of Prague, she joined the Biological Institute of the Carlsberg Foundation. She undertook research at the Harvard Medical School from 1939 to 1946, becoming a full professor in 1973. She was noted for her research on the structure of pepsin.

Sir Francis Knowles

PROFESSOR SIR FRANCIS KNOWLES, Bt, FRS, the neuroendocrinologist, died on July 13, 1974. He was 59.

Educated at the University of Oxford, he carried out pioneering work into the structure of neurosecretory systems. In 1958, he went to the Department of Anatomy of the University of Birmingham, becoming Reader in 1963 and Professor of Comparative Endocrinology in 1967. Later that year he was appointed Professor of Anatomy at the University of London and Head of Anatomy at King's College, London.

Announcements

Award

The **A. T. Shousha Foundation Medal and Prize for 1974** has been awarded posthumously to **Mohamed Taieb Hachicha.**

Appointment

Piero Sensi has been elected president of the **Societa Italiana Scienze Farmaceutiche.**

Miscellaneous

The Harkness Fellowships for study and travel in the United States—1975. Candidacy is open to men and women (between 21 and 30 years of age on September 1, 1975) in any profession or field of study provided both their secondary and further education (or equivalent professional experience in lieu of further education) have been wholly or mainly in the United Kingdom. For application forms or further information write to The Harkness Fellowships (UK), Harkness House, 38 Upper Brook Street, London W1Y 1PE.

Gottlieb–Duttweiler Prize for the promotion of nutritional sciences. Applications are invited from scientists with outstanding achievements in the field of nutrition research. Applications should be sent by December 31, 1974 to Green Meadow Foundation, c/o Institute for Nutrition Research, Seestrasse 72, CH-8803 Rüschlikon, Switzerland.

International meetings

October 24–26, **26th International Congress of Physiological Sciences,** New Delhi (Secretariat, 26th International Congress of Physiological Sciences, Department of Physiology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110016, India).

October 29–31, **5th International Symposium on Olfaction and Taste,** Parkville (Dr J. P. Coghlan, ISOT V Secretary, Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Victoria 3052, Australia).

November 4–7, **9th International Symposium on Advances in Chromatography,** Houston (Albert Zlatkis, Chemistry Department, University of Houston, Houston, Texas 77004, USA).

November 6–8, **International Symposium on Pharmacokinetics and Drug Effects, Stockholm** (Hans H. Linden, Director, Aputekarsocieten, Box 1136, S-111 81 Stockholm, Sweden).

November 7, **The Control of Cell Growth Regulation by Tumour-inducing Viruses,** The Royal Society (Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

November 10–14, **17th National Meeting, Academy of Pharmaceutical Sciences,** New Orleans (Academy of Pharmaceutical Sciences, A.Ph.A., 2215 Constitution Avenue, N.W., Washington, DC 20037, USA).

November 23, **British Academy of Psychopharmacology Open Meeting,** London (Professor Max Hamilton, President, British Academy of Psychopharmacology, University of Leeds, Department of Psychiatry, 15 Hyde Terrace, Leeds LS2 9LT, UK).

Errata

In the article "Energy problems facing India" by N. K. Sehgal (*Nature,* **249,** 710; 1974) there were serious misplacings of the textual matter.

(i) The last two paragraphs under "Genesis of problems" (page 711, starting with "A 'gobar-gas' . . .") should go at the end of "Meeting the challenge" (page 712).

(ii) The entire portion under "Fifth plan and beyond" starting with the fifth paragraph ("Petroleum products . . .") onwards, must come under "Genesis of problems" in place of the two paragraphs mentioned in (i) above.

The corrected version is being published in *Scientific Opinion,* of which Dr Sehgal is the editor, in Vol. 3, No. 2.

In the article "Antibody cell daughters can produce antibody of different specificities" by A. J. Cunningham and S. A. Fordham (*Nature,* **250,** 669; 1974) the first sentence of the legend to Fig. 1 should read, "Photographs (a–c) and diagrammatic representation (d–f) of clear (c,f) sombrero (b,e) and partial (a,d) plaques . . ."

Reports and publications

Great Britain

The Macaulay Institute for Soil Research. Collected Papers, Vol. 10, 1970/1972. Edited by A. M. B. Geddes and R. Noble. Pp. 26 + Collected Papers Nos. 1-92. (Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1974.) [127

Ordnance Survey. Professional Papers, New Series No. 26: The National Gravity Reference Net 1973 (NGRN 73). By D. M. Smith, P. M. Howell and A. B. D. E. Abernethy-Clark. (Prepared in conjunction with the Institute of Geological Sciences.) Pp. 22. (Southampton: Ordnance Survey of Great Britain, 1974.) £2. [157

Proceedings of the Royal Irish Academy. Vol. 74, Section A. No. 9: Anti-Compton Scattering. By J. L. Synge. Pp. 67–72. 18p. No. 10: Generalized Limits and Uniform Convexity. By G. de Barra. Pp. 73–78. 15p. No. 11: A Minimisation Problem with a Solution of Spline Type. By D. W. Arthur. Pp. 79–86. 20p. No. 12: Poincaré Generators for the Free Spin 3/2 Field. By G. B. Mainland. Pp. 87–90. 14p. Vol. 74, Section B. No. 9: The Namurian of West County Clare. By M. H. Rider. Pp. 125–142. 34p. No. 10: Palynological Correlations in the Cork Beds (Upper Devonian-?Upper Carboniferous) of Southern Ireland. By G. Clayton, K. Higgs, K. J. Gueinn and A. Van Gelder. Pp. 145–156 + plates 3 and 4. 38p. No. 11: Condensation of Aromatic Aldehydes with 5-Amino-4-Cyano-3-Cyanomethylpyrazoles. By C. N. O'Callaghan. Pp. 157–160. 14p. No. 12: A Late-Midlandian Section at Finglas River, near Waterville, Kerry. Pp. 161–178. 28p. No. 13: A Tertiary Dyke System in South-West Ireland. By P. Morris. Pp. 179–184. 15p. (Dublin: Royal Irish Academy, 1974.) [157

Bulletin of the British Museum (Natural History). Geology. Vol. 25, No. 1: The Ornithischian Dinosaur *Hypsilophodon* from the Wealden of the Isle of Wight. By P. M. Galton. Pp. 1–152 + 2 plates. £7.35. Vol. 25, No. 2: The Taxonomy and Morphology of *Puppigerus camperi* (Gray), an Eocene Sea-Turtle from Northern Europe. By Richard Thomas Jones Moody. Pp. 153–186 + 8 plates. £2.85. Zoology. Vol. 26, No. 4: The Indigenous Earthworms (Megascolecidae: Oligochaeta) of Tasmania. By B. G. M. Jamieson. Pp. 201–328 + 10 plates. £6.75. Vol. 26, No. 5: The Freshwater Fishes of Rivers Mungo and Meme and Lakes Kotto, Mboandong and Soden, West Cameroon. By E. Trewavas. Pp. 329–419 + 5 plates. £5.60. Vol. 26, No. 6: Notes on some Echinoderms from Southern Africa. By A. M. Clark. Pp. 421–487 + 3 plates. £3.40. (London: British Museum (Natural History), 1974.) [167

Energy and the Environment. (Report of a Working Party set up jointly by Committee for Environmental Conservation; Royal Society of Arts; and the Institute of Fuel.) Pp. 45. (London: The Royal Society of Arts, 1974.) £3. [167

Sharks and Rays. (Introductory Leaflet). Pp. 6. (London: British Museum (Natural History), 1974.) 4p. [177

Bulletin of the British Museum (Natural History). Entomology. Vol. 30, No. 7: A Revision of the Genus *Passeromyia* Rhodhain and Villeneuve (Diptera: Muscidae). By A. C. Pont. Pp. 339–372. £1.80. Zoology. Vol. 27, No. 1: Anatomy of Head and Neck in the Huia (*Heteralocha acutirostris*) with Comparative Notes on Other Callaeidae. By P. J. K. Burton. Pp. 1–48 + 1 plate. £2.30. (London: British Museum (Natural History), 1974.) [177

The Honeybee. (Entomology Leaflet No. 6). Pp. 8. (London: British Museum (Natural History), 1974.) 7p. [187

Other Countries

Consiglio Nazionale delle Ricerche, Laboratorio de Stude Sulla Ricerca e Sulla Documentazione. Note di Bibliografia e di Documentazione Scientifica. Scheme per una rete di Informatica nel Settore della Documentazione Scientifico-Tecnica: Elementi Preliminari. By Paolo Bisogno. Pp. 80. Indici Logica e Linguaggio: Problemi di Catalogazione Semantica. By Alfredo Serrai. Pp. 95. (Roma: Consiglio Nazionale delle Ricerche, 1972 and 1974.) [157

World Health Organization. Technical Report/Series. No. 544: Uses of Epidemiology in Housing Programmes and in Planning Human Settlements—Report of a WHO Expert Committee on Housing and Health. Pp. 64. 6 Sw. francs. No. 545: Pesticide Residues in Food—Report of the 1973 Joint FAO/WHO Meeting. Pp. 42. 5 Sw. francs. No. 547: The Planning of Medical Education Programmes—Report of a WHO Expert Committee. Pp. 25. 4 Sw. francs. (Geneva: WHO; London: HMSO, 1974.) [157

Académie Royale de Belgique. Mémoires de la Classe des Sciences. 2e Serie, T.XLI, Easc. 2: l'Ostéologie d'*Elops* Linné, C., 1766 (Pisces Elopiformes) et son Intérêt Phylogénétique. Par L. Taverne. Pp. 96. (Bruxelles: Académie Royale de Belgique, 1973). [157

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